The capability of methanoarchaea to form methyl and hydride derivatives of metals and metalloids

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“Mathematicians can prove things – according to one strict view,
they are the only people who can – but the best that scientists can do
is fail to disprove things while pointing to how hard they tried.”
(Richard Dawkins; “The Greatest Show on Earth” 2009)

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1. Introduction

1.1. Theoretical background

This thesis focuses on the formation of methyl and hydride metal(loid) compounds by methanoarchaea. The metal bismuth (Bi), the metalloids antimony (Sb), arsenic (As) and tellurium (Te) as well as the non-metal selenium (Se) - hereinafter referred to as metal(loid)s - are covered as representatives of the International Union of Pure and Applied Chemistry (IUPAC) periodic table Group 15 and 16 elements. The abundance of these metal(loid)s in the earth's crust is relatively low, ranging from 2-5 ppm for arsenic to 1-10 ppb for the other elements. However, the concentration of these elements can locally vary e.g. by differences in the natural contribution and by anthropogenically accumulation in the course of increasing use in technical and medical applications.

A comprehensive overview of elements subjected to biomethylation was given by John S. Thayer (Tab. 1.1). The methylation and hydride generation notably modify the mobility of metal(loid)s. For instance, methyl and hydride derivatives of metal(loid)s are more hydrophobic than their precursor (Thayer 1988; Thayer 2002). To rationalize the increased hydrophobicity caused by metal(loid) methylation and hydride generation it is useful to compare the electronegativity of the metal(loid)s and the elements typically forming stable compounds with these metal(loid)s (Tab. 1.2). In most cases, the carbon of a methyl group or hydrogen replaces oxygen of a metal(loid) oxyanion, resulting in a less polar bond as indicated by a smaller difference between the electronegativity values.

Tab. 1.1: Elements reportedly biomethylated according to John S. Thayer (Thayer 2002); modified.

Periodic group number								
9	10	11	12	13	14	15	16	17
				Al	Si	P	S	Cl
				NR	NR	1	1-4	2,3
Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br
1-4	1	NR	NR	NR	1(?)	1-4	1-4	2,3
			Cd	In	Sn	Sb	Te	I
			1(?)	NR	1,2	1,2	1,2,4	1-3
			Hg	Tl	Pb	Bi	Po	At
			1	1	1,2(?)	1	1,2	NR

(NR) not reported (1) bacteria and archaea (2) fungi/algae/yeast (3) plants (4) animals

Tab. 1.2: Electronegativity according to Pauling for selected elements (Atkins et al. 2010).

Element	Symbol	Atomic number	Atomic weights	Periodic group	Electronegativity
Hydrogen	H	1	1.01	1	2.2
Carbon	C	6	12.01	14	2.55
Arsenic	As	33	74.92	15	2.18
Antimony	Sb	51	121.76	15	2.05
Bismuth	Bi	83	208.98	15	2.02
Oxygen	O	8	16.00	16	3.44
Sulfur	S	16	32.06	16	2.58
Selenium	Se	34	78.96	16	2.55
Tellurium	Te	52	127.60	16	2.1

Permethyl and hydride derivatives of the covered metal(loid)s exhibit also a high vapor pressure over the condensate phase, leading to a notable increase of these compounds in the gas phase at room temperature and is thus referred to as volatilization. A better understanding of biomethylation and hydride generation processes is also important for an improved health and environmental risk management as the methylation and hydride generation results in a modulation of the toxicity of the metal(loid) derivatives.

1.1.1. The research on organometal(loid) compounds: A historical overview

Occurrence of metal(loid) methyl and hydride derivatives was discovered in several - mainly anaerobic - habitats like landfills, sewage sludge fermentation plants, geothermal springs, alluvial soils and the gut of mice and man (Feldmann and Hirner

1995; Hirner et al. 1998; Michalke et al. 2000; Meyer et al. 2007; Meyer et al. 2008; Michalke et al. 2008; Diaz-Bone and Van de Wiele 2009). The formation of methyl and hydride metal(loid) compounds is thereby usually caused by microbial activity. Comprehensive reviews on this issue were given by Ronald Bentley and Thomas G. Chasteen (Bentley and Chasteen 2002; Chasteen and Bentley 2003).

The first documented report of volatile metal(loid)s goes back to a dissertation by Caroli de la Font in the 17th century but was rejected due to "absurd conjectures and reasoning" (Bentley and Chasteen 2002). In the beginning of the 19th century, poisonings in Germany caused by wallpaper paintings containing arsenic pigments spread. The chemist Leopold Gmelin suggested in 1839 that the poisonings were caused by volatilization of arsenic pigments used. At the end of the 19th century, the works of the Italian physician Bartolomeo Gosio demonstrated that certain fungi were responsible for the proposed volatilization of arsenic, forming the so-called "Gosio Gas". Frederick Challenger identified "Gosio Gas" as trimethylarsine ($(CH_3)_3As$) a few years later (Challenger 1945).

In 1971, the formation of dimethylarsine ($(CH_3)_2AsH$) by crude extracts prepared from a methanoarchaeum was demonstrated (McBride and Wolfe 1971). This pioneering work entailed numerous studies that underpinned the distinguished capability of methanoarchaea to volatilize metal(loid)s by methylation and hydride generation (Michalke et al. 2000; Michalke et al. 2002; Meyer et al. 2007; Meyer et al. 2008). Motivated by the discovery of methanoarchaea in the human gut (Miller et al. 1982; Miller and Wolin 1985; Miller and Wolin 1986; Hackstein et al. 1995; Eckburg et al. 2005), the formation of volatile, mainly methylated metal(loid) derivatives by human feces and representatives of the human gut microbiota was demonstrated in the recent years (Meyer et al. 2008; Michalke et al. 2008; Diaz-Bone et al. 2009; Diaz-Bone and Van de Wiele 2009; Diaz-Bone and Van de Wiele 2010). These findings are currently discussed in terms of pathogenic effects of methanoarchaea inhabiting the human gut.

1.1.2. Use of metal(loid)s and their toxicity

1.1.2.1. *Arsenic*

Arsenic naturally occurs mainly as a component of sulfidic ores like arsenopyrite (FeAsS) (Tamaki and Frankenberger 1992). The extensive use of arsenic

compounds in agriculture e.g. in herbicides lead to an increase of arsenic in soils. From there arsenic can enter the ground- and freshwater by leaching processes. For instance, cacodylic acid ($(CH_3)_2AsO(OH)$) is widely used is arsenic containing pesticides. It became infamous under the codename “agent blue” used by the US Army during the Vietnam War. Agent blue was sprayed on crops, causing them to dry out and thus depriving the Viet Cong of their food resources (Stellman et al. 2003).

The predominant form of arsenic in oxidized water is arsenate ($AsO(OH)_3$) and in anoxic water the more toxic trivalent arsenite ($As(OH)_3$). Arsenate readily enters cells via phosphate transport systems by mimicking phosphate. Under anaerobic conditions, arsenate is reduced to the more toxic trivalent arsenite in the cells which strongly binds to thiol groups of proteins (Paez-Espino et al. 2009).

Exposure to high arsenic concentration is e.g. reported in Bangladesh and West Bengal (Rahman et al. 2001). The arsenic exposure to humans originates from tube wells connected to the highly contaminated groundwater. This exposure results in numerous cases of arsenicosis (arsenic intoxication). The first notable symptoms of arsenicosis are typical skin lesions. The long term exposure to high arsenic concentrations is finally lethal.

Regarding methyl and hydride arsenic derivatives, several acute toxic and carcinogenic effects have been assigned to trivalent methyl and hydride arsenic derivatives including volatile $(CH_3)_3As$ and arsine (AsH_3) (Styblo et al. 2002; Andrewes et al. 2003). AsH_3 in particular causes hemolysis by oxidizing the heme, leading to an elevated formation of membrane damaging decomposition products (Rael et al. 2006). The higher cytotoxic effects of trivalent methylated arsenic derivatives relative to inorganic arsenite and arsenate as well as pentavalent methylated arsenic derivatives have been demonstrated on Chinese hamster ovary cells *in vitro* (Dopp et al. 2004a; Dopp et al. 2004b). The involvement of the human intestinal microflora in the formation of toxic arsenical derivatives was recently shown (Diaz-Bone et al. 2009; Diaz-Bone and Van de Wiele 2010). Consumption of highly contaminated rice is currently discussed as an important source of arsenic (Meharg et al. 2009; Zhao et al. 2010). The consumed arsenic can then be converted by the human gut microbiota into more toxic derivatives.

1.1.2.2. *Selenium*

The natural levels of selenium vary locally. It can be found in copper sulfide ores (Atkins et al. 2010). In a certain range of concentration, selenium is a verifiable nutrition in contrast to the other elements covered in this thesis (Chasteen and Bentley 2003). Selenium can replace sulfur in cysteine to form the "21st proteinogenic amino acid" selenocysteine. Besides its use as dietary supplement, selenium is also used in numerous technical applications like in photoelectrical cells and in semiconductors. Selenium plays also an important role in medical applications e.g. in protective agents against neurotoxicity.

Exposure to high selenium concentrations, however, leads to selenosis (selenium intoxication). The symptoms of selenosis are dermatitis, loose hair, diseased nails and peripheral neuropathy. Studies on methylated selenium derivatives suggest a lower toxicity than of inorganic species. Hence, methylation of selenium is hitherto considered a detoxification mechanism (Chasteen and Bentley 2003) as well as the transformation of selenium derivatives to elementary Se^0.

1.1.2.3. *Antimony*

Natural concentrations of antimony in water are relatively low (<1 µg L^{-1}) (Filella et al. 2007). Only in geothermal springs, concentrations of up to 1 g L^{-1} can occur. Its most common forms in the environment are antimonate ($Sb(OH)_6^-$) and antimonite ($Sb(OH)_3$). Antimony is e.g. used in semiconductor technology, in antifriction alloys, in batteries etc. (Filella et al. 2002). It is also used in fire-protective additives.

Antimony is accessible to microbial volatilization by methylation and hydride generation processes (Michalke et al. 2000; Wehmeier and Feldmann 2005). Volatile methyl and hydride antimony derivatives - referred to as stibine - were considered to be highly toxic and were brought into connection with several cases of sudden infant death syndromes (SIDS). However, no experimental evidences could affirm this assertion hitherto (Jenkins et al. 1998; Filella et al. 2007). Only few toxicological studies concerning methylated antimony compounds are available yet. In analogy to arsenic, trivalent antimony compounds are more toxic than pentavalent compounds. Regarding volatile antimony derivatives, DNA damage caused by high concentrations (200 µmol L^{-1}) of stibine (SbH_3) and trimethylstibine ($(CH_3)_3Sb$) *in vitro* has been reported (Andrewes et al. 2004).

1.1.2.4. *Tellurium*

Tellurium is in many aspects similar to selenium. However, no nutritional requirement of tellurium is known so far (Ogra 2009). Tellurium is mainly used in alloys and semiconductor technology (Chasteen and Bentley 2003).

The soluble oxyanions of tellurium are more toxic than the insoluble elemental tellurium (Chasteen and Bentley 2003). Microbial reduction of tellurite to elemental tellurium is hence considered as a resistance mechanism. Toxicological data for human exposure are still scarce.

Microbial methylation of tellurium is widespread in the environment (Chasteen and Bentley 2003). Tellurium methylation results in the formation of volatile compounds with “abominable” garlic like odor.

1.1.2.5. *Bismuth*

Bismuth is only found in its trivalent oxidation state in the environment e.g. as bismite (Bi_2O_3) or bismuthinite (Bi_2S_3). Pentavalent bismuth derivatives are in contrast to the other elements of Group 15 strong oxidation agents in aqueous solution and thus not stable under normal environmental conditions (Filella 2010). Inorganic bismuth is considered non-toxic for humans but toxic for some prokaryotes like *Helicobacter pylori*. Due to the low toxicity of bismuth for humans it is widely used e.g. in cosmetics as pigment, for low-melting alloys, in semiconductor technology and in drugs against gastrointestinal disorders, syphilis and dermatological disorders. Colloidal bismuth subcitrate (CBS) was and still is in some countries extensively used as anti peptic ulcer drug against irritable bowel syndrome and against *Helicobacter pylori* infection (Diaz-Bone and Van de Wiele 2010).

However, recent studies recommend caution in extended use of bismuth as genotoxic effects were indicated (Asakura et al. 2009). The mainly neurotoxic effects of volatile methylated trimethylbismuth ($(CH_3)_3Bi$) on mammals were intensively analyzed by Sollmann and Seifter 1939 (Sollmann and Seifter 1939). Several cases of neurotoxic effects in patients treated with inorganic bismuth salts were also reported in France in the 1970s (Dopp et al. 2004a). The cause of these toxic effects, however, remained unsolved. Toxicological trials pointed towards induction of cyto- and genotoxic effects of methylated bismuth compounds (von Recklinghausen et al. 2008). Besides the indicated direct toxic effects of methylated bismuth compounds on humans also indirect effects were suggested from inhibitory effects of $(CH_3)_3Bi$ on

Bacteroides thetaiotaomicron, a representative of the human physiological intestinal microflora (Meyer et al. 2008).

1.1.3. State-of-the-art of metal(loid) methylation and hydride generation

Numerous anthropogenic methyl group donors are known. In terms of biological metal(loid) methylation it turned out that S-adenosly methionine (SAM) and methylcobalamin ($CH_3Cob(III)$) (Fig. 1.1) are the most relevant (Bentley and Chasteen 2002; Thayer 2002). Despite the numerous reports of microbial formation of metal(loid) hydrides like arsine, stibine and bismuthine in the environment or by pure culture experiments, details of biological hydride generation of metal(loid)s are still lacking. In terms of chemical hydride derivatization for analytical applications, sodium tetrahydroborate is the best analyzed and most used agent today (D'Ulivo 2004).

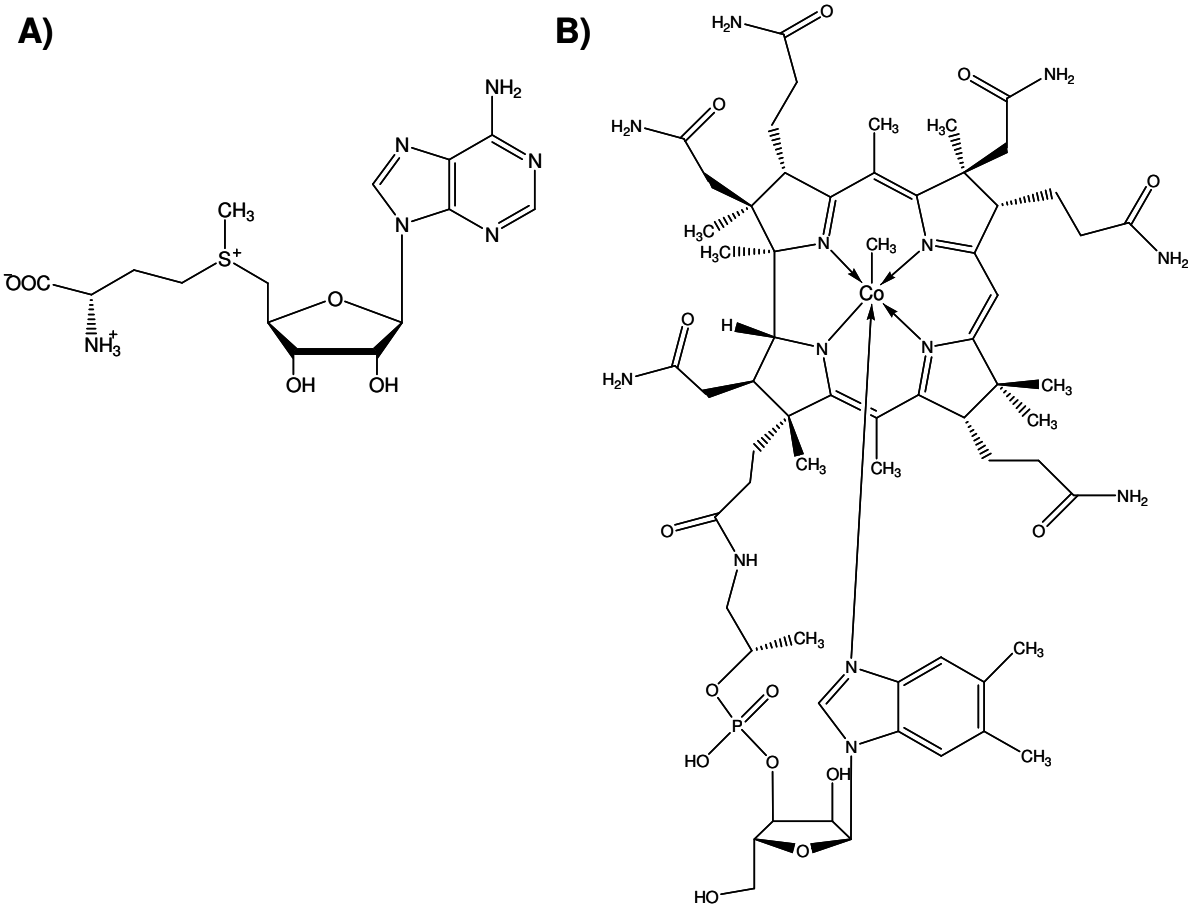

Fig. 1.1: Structure formula of S-adenosyl methionine A) and methylcobalamin B).

1.1.3.1. *The proposed mechanism for SAM dependent metal(loid) methylation*

The first mechanistic metal(loid) methylation studies, concerning the methylation of arsenate ($AsO(OH)_3$) by the fungus *Scopulariopsis brevicaulis*, were performed by Frederick Challenger and his coworkers (Bentley and Chasteen 2002). SAM was identified as the methyl donor in the analyzed methylation reactions of arsenic. The mechanistic of forming $(CH_3)_3As$ from $AsO(OH)_3$ with SAM as the methyl donor was formulated as following (see Fig. 1.2):

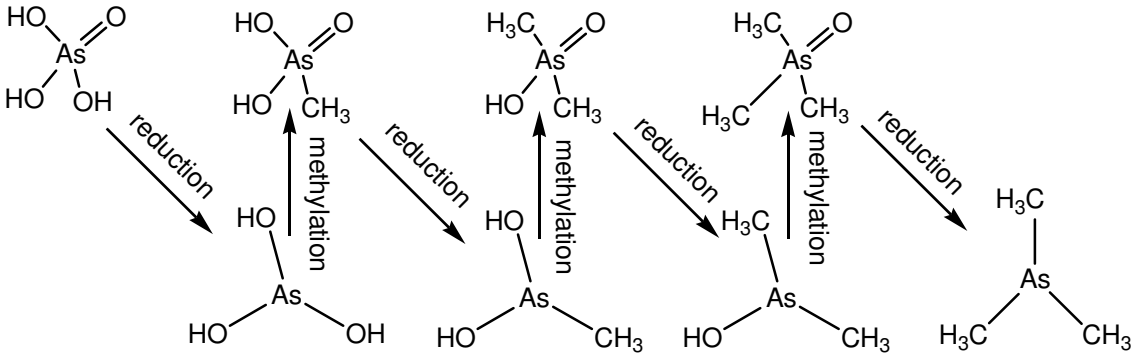

Fig. 1.2: Schematic of the "Challenger Mechanism" (Challenger 1945).
The mechanism illustrates the proposed oxidative methylation of arsenate ($AsO(OH)_3$) to trimethylarsine $As(CH_3)_3$.

Initially, $AsO(OH)_3$ is reduced in a two-electron reduction step forming $As(OH)_3$. $As(OH)_3$ is then methylated by a methyl carbocation via a nucleophilic attack of arsenic upon the methyl group of SAM, forming $CH_3AsO(OH)_2$ and S-adenosyl homocysteine. $CH_3AsO(OH)_2$ is then again reduced to form $CH_3As(OH)_2$ prior the next methylation step. This alternation can be continued until the last methylation step forms the soluble $(CH_3)_3AsO$. A last reduction step leads to the formation of volatile $(CH_3)_3As$. The involvement of thiols like glutathione (GSH) was suggested for the two-electron reduction steps of the pentavalent arsenic compounds. This mechanism became known as the "Challenger Mechanism".

More recent studies of enzymatic arsenic methylation suggest that only in the initial step $AsO(OH)_3$ is reduced, followed by substitution of the hydroxyl groups with thiol groups like GSH, forming $As(GS)_3$ in an equilibrium reaction. By a nucleophilic attack of $As(GS)_3$ upon the methyl carbocation of SAM via a lone-pair of one thiol, $CH_3As(GS)_2$ is formed (Hayakawa et al. 2005) (Fig. 1.3). The formed $CH_3As(GS)_2$ is preserved in its trivalent state and further methylated or it forms $CH_3As(OH)_2$ which can oxidize to $CH_3AsO(OH)_2$. The pentavalent methyl arsenicals represent a metabolic end product and not an intermediate of a Challenger-like methylation

pathway. The SAM dependent methylation of arsenic is catalyzed by a methyltransferase designated As3MT, Cyt19 or ArsM (Hayakawa et al. 2005; Qin et al. 2006; Thomas et al. 2007). Homologous genes potentially encoding this methyltransferase are found in bacteria and archaea (e.g. *Rhodopseudomonas palustris* and *Methanosarcina mazei*) as well as in mammals like rats and humans.

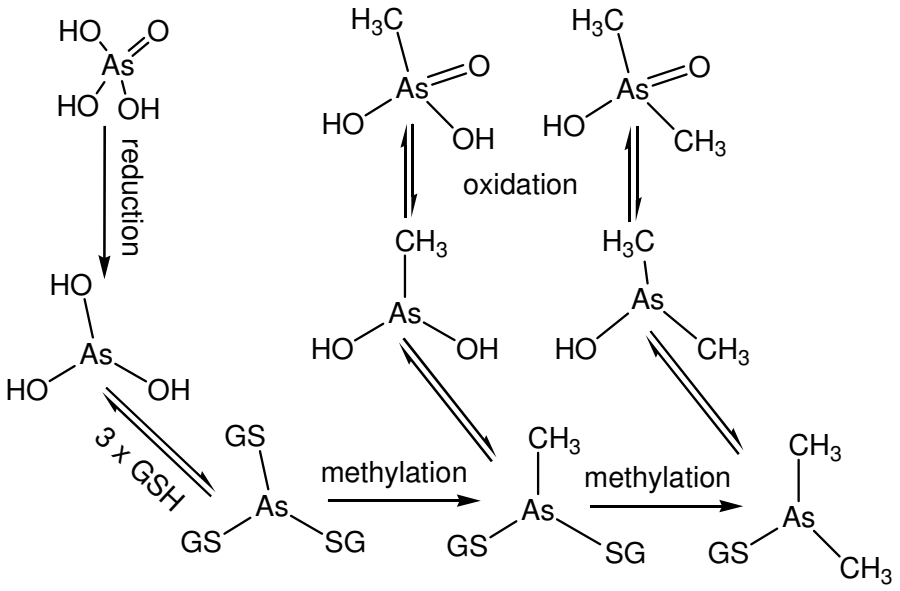

Fig. 1.3: Schematic of arsenic methylation as proposed by Toru Hayakawa and coworker (Hayakawa et al. 2005).
The methylation reaction is catalyzed by SAM-dependent methyltransferase Cyt19. GSH: Glutathione, GS: Glutathione-moiety.

The involvement of SAM as the methyl donor was also suggested for selenium, antimony and tellurium. However, experimental data support this assertion only for selenium and antimony methylation by *S. brevicaulis* (Bentley and Chasteen 2002; Chasteen and Bentley 2003).

1.1.3.2. *Methylcobalamin, a methyl donor with high versatility*

In contrast to SAM, which is an obligatory methyl carbocation donor, $CH_3Cob(III)$ can theoretically provide its methyl group as (i) a carbocation, (ii) a radical or (iii) a carbanion (Ridley et al. 1977b; Thayer 2002). Numerous studies suggested transfer of carbocations from $CH_3Cob(III)$ in physiological relevant transmethylation reactions like the methionine synthesis from homocysteine and the HS-CoM methylation in methanogenesis (LeClerc and Grahame 1996; Banerjee and Ragsdale 2003). That $CH_3Cob(III)$ can also act as a methyl donor for arsenic, bismuth and mercury methylation was shown in experiments using cell crude extracts of methanoarchaea

or sulfate reducing bacteria (SRB), respectively (McBride and Wolfe 1971; Choi et al. 1994a; Michalke et al. 2002). Though enzymatic metal(loid) methylation by $CH_3Cob(III)$ covers only assays with cell crude extracts hitherto, numerous abiotic studies provide indications for the underlying methylation mechanism.

Some studies suggested metal(loid) methylation by carbanions from $CH_3Cob(III)$. For instance, it was demonstrated that Hg(II) abstracts a methyl carbanion from $CH_3Cob(III)$ by an electrophilic attack of the former on the latter (Bertilss and Neujahr 1971). A similar methylation was proposed for other elements (Schrauzer 1974; Ridley et al. 1977a; Ridley et al. 1977b). Schrauzer and coworker reported the formation of AsH_3, CH_3AsH_2, $(CH_3)_3As$ as well as traces of $(CH_3)_2AsH$ and CH_4 when As_2O_3 was incubated anaerobically with $CH_3Cob(III)$ (or its analog methylcobaloxime) and dithioerythritol (DTE) or Zn/NH_4Cl as reductants (Schrauzer et al. 1973). Also formation of $(CH_3)_2Se$ from SeO_2 was demonstrated under the same conditions. It was suggested that the methyl carbanion formation was promoted by an increase of the electron density at the $CH_3Cob(III)$ under formation of a disulfide. The disulfide should be formed by two thiols coordinated towards the corrinoid $CH_3Cob(III)$ (Fig. 1.4). The methyl carbanion then methylates the trivalent arsenic in a non-oxidative methylation step.

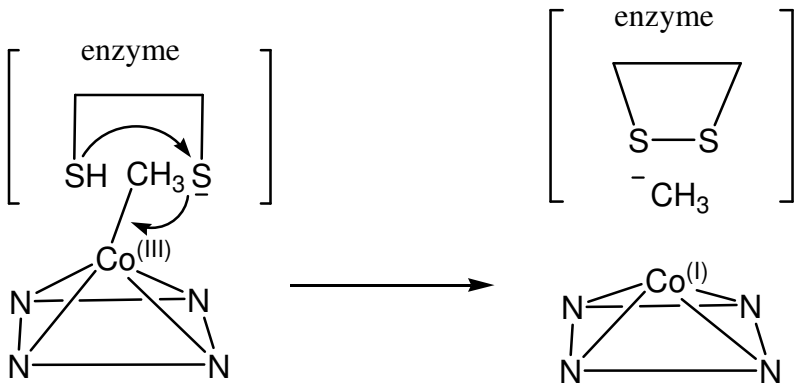

Fig. 1.4: Proposed mechanism of the formation of a "cryptocarbanion" from $CH_3Co(III)$ according to Gerhard N. Schrauzer and coworker (Schrauzer et al. 1973).
An enzyme bound carbanion is formed upon reductive cleavage of $CH_3Cob(III)$. Electrons for the reductive cleavage are provided upon formation of a disulfide.

This mechanism was later rejected by some authors because (i) a coordination of thiols to $CH_3Cob(III)$ could not be shown by ^{13}C and ^{1}H NMR studies (Ridley et al. 1977a) and (ii) the reductive conditions in the performed assays would not favor the proposed mechanism (Zakharyan and Aposhian 1999). Why the mechanism proposed by Schrauzer and coworker is unlikely under reductive conditions was,

however, not explained. Instead, a nucleophilic attack of arsenite on $CH_3Cob(III)$ in analogy to the “Challenger Mechanism” was preferred, resulting in an oxidative methylation with subsequent requirement of reduction of the formed pentavalent arsenical by GSH.

Some studies also postulated metal(loid) methylation by methyl radicals formed upon homolytic cleavage (Ridley et al. 1977b). It was suggested that elements with redox potential lower than +0.6 V are methylated by a methyl radical like e.g. tin. In the presence of a single-electron oxidant like Fe(III), Se(II) is oxidized to form a Se(III) radical which then cleaves $CH_3Cob(III)$ homolytically, resulting in $CH_3Se(II)$ derivative and Cob(II). Reactive methyl radicals can also be formed by photolytical cleavage of $CH_3Cob(III)$ (Hogenkamp 1966).

1.1.3.3. *Metal(loid) methylation as a proposed side reaction of basal cell metabolism*

Due to the reported involvement of $CH_3Cob(III)$ in metal(loid) methylation, some authors assumed that metal(loid) methylation is in some cases a side reaction of $CH_3Cob(III)$-dependent physiological pathways. A link between central $CH_3Cob(III)$-dependent reaction of methanogenesis and metal(loid) methylation was suggested due to the high content of $CH_3Cob(III)$ in methanoarchaea and the finding that all methanoarchaea tested are capable to methylate metal(loid)s (Krzycki and Zeikus 1980; Meyer et al. 2008). In the case of mercury methylation by SRB, tracer experiments with radiolabeled ^{14}C compounds as well as specific inhibitor studies indicated that Hg methylation by *Desulfovibrio desulfuricans* is connected to the acetyl coenzyme A synthase pathways, utilizing $CH_3Cob(III)$ as methyl carrier (Choi et al. 1994b). As this pathway is not only restricted to SRB, Hg methylation via this pathway might be widespread among various procaryotes.

Various pathways of selenium methylation like the methionine/seleno-methionine synthase pathway have been recognized in microorganisms and plants (Chasteen and Bentley 2003). Selenium is incorporated into amino acids or other metabolites either by specific enzymes or by enzymes not distinguishing between sulfur and selenium. Volatile methyl or hydride species of selenium might be formed by degradation processes of seleno-amino acids in analogy to the formation of volatile organosulfur compounds. A methyltransferase not involved in the metabolism of seleno methionine or selenocysteine has also been identified to promote the

volatilization of selenium in broccoli (Zhou et al. 2009). This methyltransferase is apparently mainly involved in the ubiquinone biosynthesis.

1.1.4. Methanoarchaea: A physiological group with unusual features

Methanoarchaea belong to the domain of archaea. In fact, methanoarchaea were the first archaea identified as such (Fox et al. 1977; Woese et al. 1978). Like bacteria, archaea are prokaryotes lacking a real cell nucleus and organelles like mitochondria which are typical for eucaryotes. Archaea share some further characteristics with bacteria like their size (about 0.1 to 2 µm), their simple shape (spherical cocci or rod like), their physiological versatility and their genome organization in a circular DNA (Brown and Doolittle 1997). However, sequence analyses of the ribosomal RNA suggested that archaea are phylogenetically remote to bacteria and eucarya. Also in some aspects of biochemistry like the composition of the cell wall and cell membrane, cofactors and information processing (DNA replication and transcription) etc., archaea are different from bacteria.
Numerous reviews dealing with the unique ecology, phylogeny and biochemistry are available (refer to (Ferry 1992; Thauer 1998; Deppenmeier 2002; Deppenmeier 2004; Thauer et al. 2008). A brief summary about methanogens in accordance to the named reviews is given in the following sections.

1.1.4.1. *Where they live and what they do*

Methanoarchaea yield their energy from a unique metabolic pathway which results in the formation and subsequently release of methane (CH_4). They are widespread in anaerobic habitats like fresh-water sediments of lakes and rivers, swamps, tundra, rice paddies, anaerobic digesters of sewage plants, in the intestine of termites, ruminants and in humans (Miller and Wolin 1986; Deppenmeier 2002). The formation of methane by these organisms in their habitat is dependent on the availability of acetate, methylated amines ($(CH_3)_3N$, $(CH_3)_2NH$, CH_3NH_2) or methanol (CH_3OH). The reduction of these metabolic end products of acetogenic and syntrophic bacteria to methane represents the last step in the anaerobic food chain and plays therefore an important part in the global carbon cycle. Methanogens also contribute to the H_2 consumption during anaerobic processes by using H_2 to reduce CO_2 to methane. However, methanoarchaea are competed by SRB in sulfate-rich habitats.

The formation of methane is of great ecological importance as methane is one of the most potent greenhouse gases. The anthropogenic contribution to the global methane release is linked to the intensive animal husbandry of ruminants. In addition, involvement of methanoarchaea in the formation of methane gas hydrates found in high abundance in ocean floor sediments is discussed. Both, environmental risk by uncontrolled release as well as high potential of solving fuel problems arise from these huge methane deposits. Another environmentally important capability of methanoarchaea is the detoxification of xenobiota like halogenated compounds. Studies are available which demonstrate the dehalogenation of trichlorofluoromethane by *M. barkeri* (Krone and Thauer 1992).

1.1.4.2. *The five orders of methanoarchaea*

Methanoarchaea are classified into five phylogenetical orders: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanopyrales* and *Methanosarcinales*. Apart from the same principal of energy metabolism, the members of the particular orders are phylogenetically remote. Members of the first four orders are all restricted to methanogenesis from CO_2 and H_2 or formiat and H_2 and are therefore called "hydrogenotroph". *Methanosphaera stadtmaniae* take a special position as it reduces methanol (CH_3OH) in a H_2-dependent pathway. Among the members of the first four orders psychrophilic (*Methanogenium frigidum*), thermophilic (e.g. *Methanothermobacter marburgensis*) and even hyperthermophilic (e.g. *Methanococcus janashii* and *Methanopyrus kandleri*) organisms are found. Nevertheless, most methanoarchaea known today are mesophilic with an optimal growth temperature between 30 - 37 °C.

Members of the order *Methanosarcinales* are the most versatile regarding their methanogenic substrates. With only a few exception, all members of this order can utilize H_2 + CO_2, CH_3OH, $(CH_3)_3N$, $(CH_3)_2NH$, CH_3NH_2 and methylthiols in the so-called methylotrophic methanogenesis and acetate (CH_3COOH) in the acetoclastic branch of methanogenesis.

1.1.4.3. *The cofactors and prosthetic groups of methanogenesis*

The biochemistry of methanogenesis requires some unusual cofactors and prosthetic groups. These cofactors and prosthetic groups can be divided into C_1 carrier and electron carrier.

The most important C_1 carriers are the vitamin B_{12} derivative methylcobalamin ($CH_3Cob(III)$) (Fig. 1.1), methanofuran (MFR), tetrahydromethanopterin (H_4MPT) and 2-mercaptoethanesulfonate (HS-CoM) (Fig. 1.5).

The list of typical electron carriers comprises the deazaflavine F_{420}, nicotinamide adenine dinucleotide phosphate ($NADP^+$), N-7-mercaptoheptanoyl-L-threonine phosphate (HS-CoB) and methanophenazine (Fig. 1.6). Methanophenazine is only found in the order *Methanosarcinales*, which also harbors cytochromes (Thauer et al. 2008). Further differences between cofactors of different methanoarchaea were observed. For instance in *Methanosarcinales* some side chains of the cofactors tent do exhibit additional glutamates like in the case of H_4MPT, hence called tetrahydromethansarcinapterin (H_4SPT). In the following section, the principal reaction steps leading to methane formation and the build-up of a transmembrane proton and sodium gradient will be described.

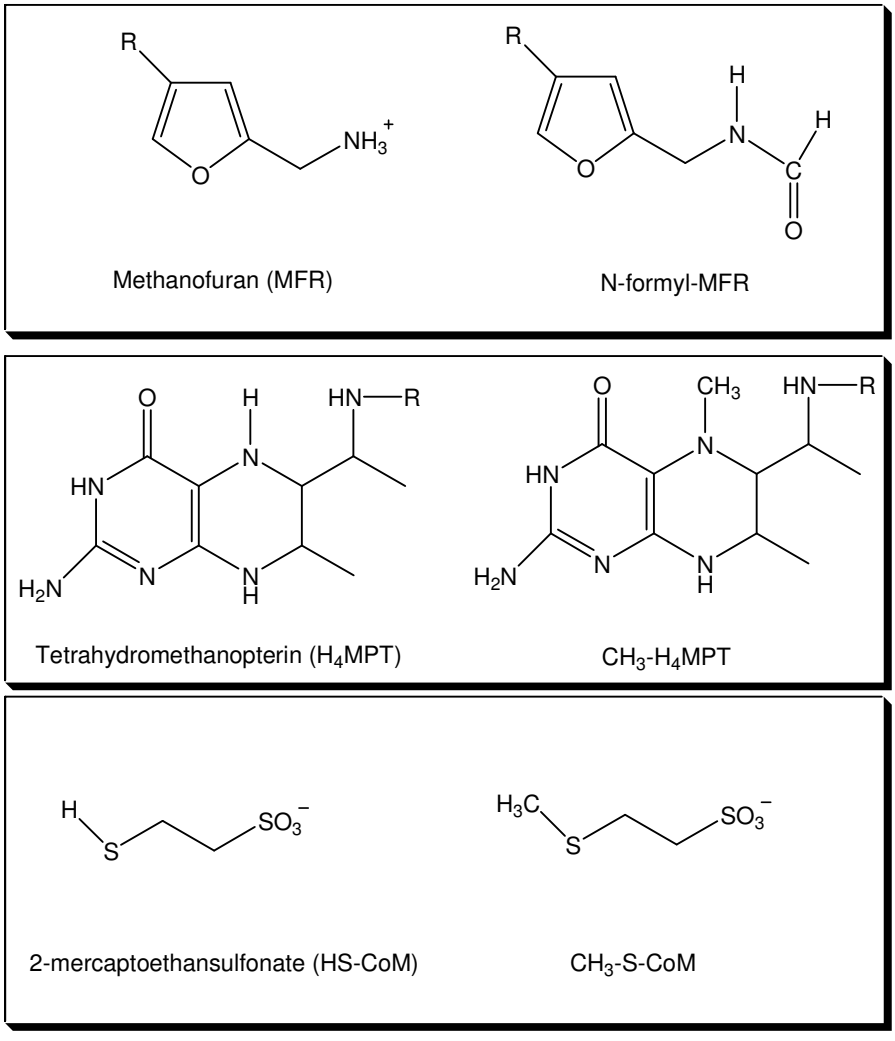

Fig. 1.5: Important C1-carrier involved in methanogenesis.
R: moiety

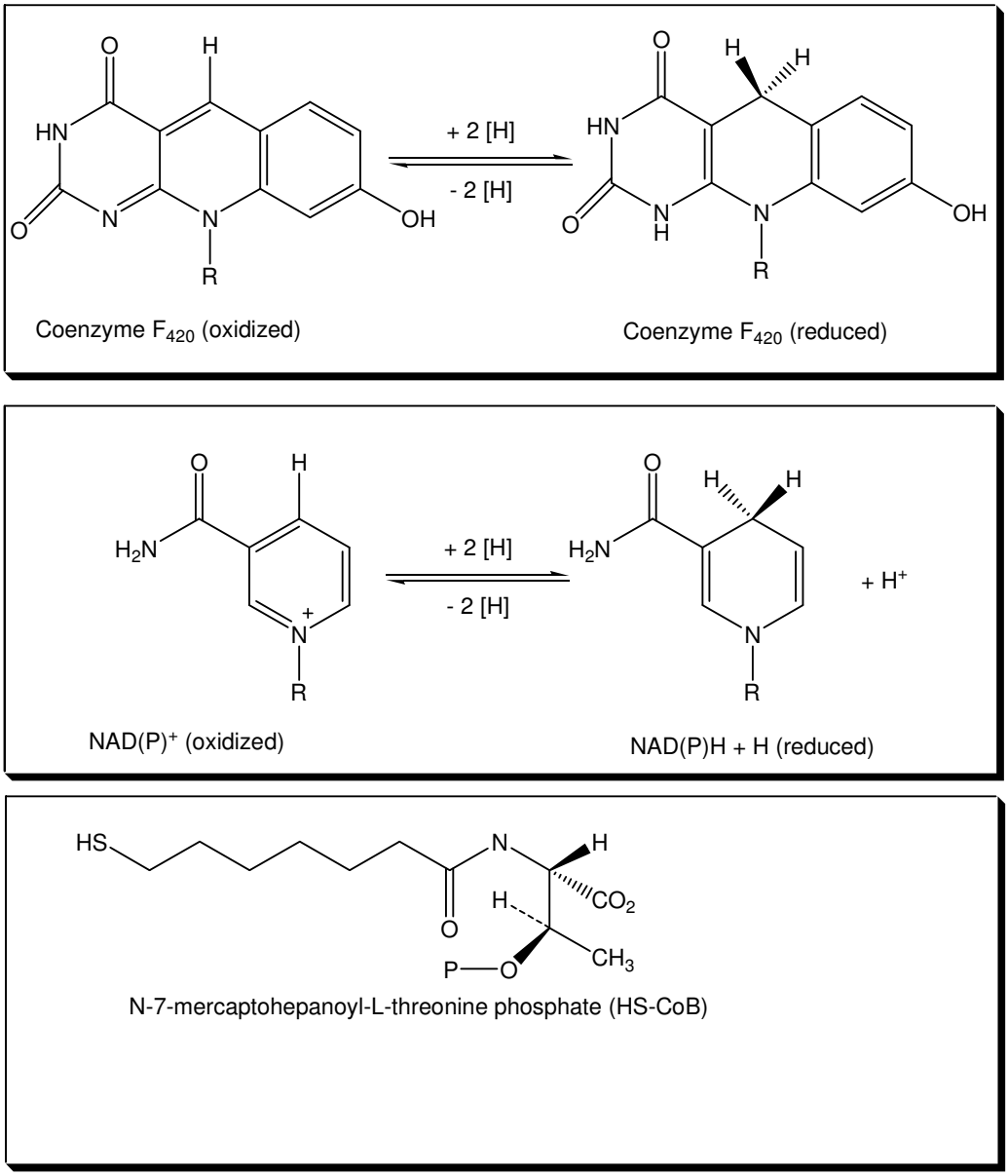

Fig. 1.6: Important electron carrier of methanogenesis.
R: moiety

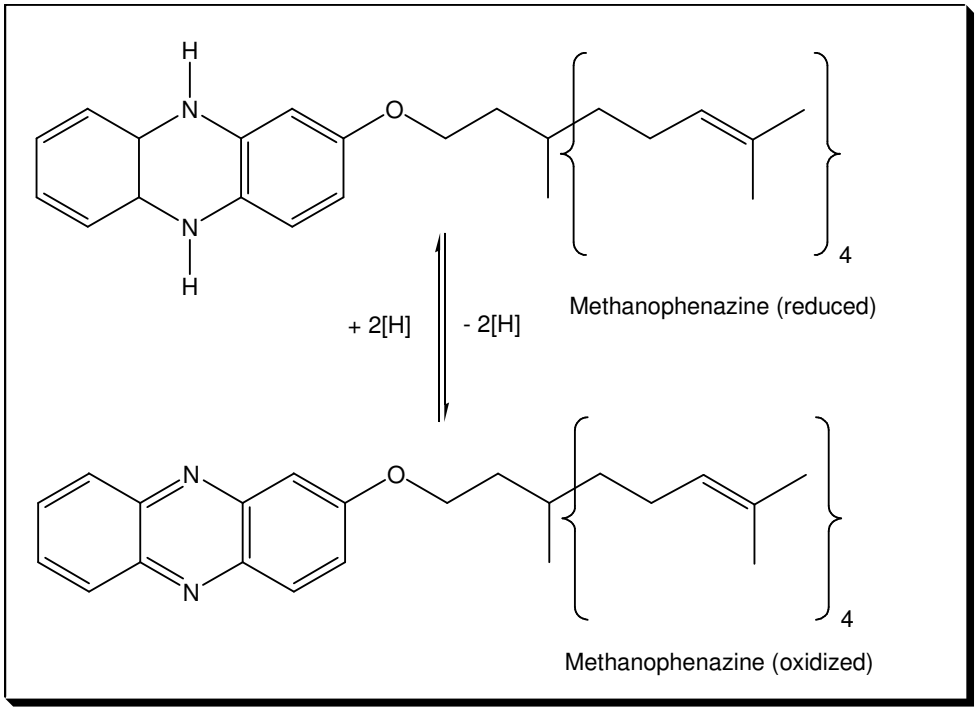

Fig. 1.6: Important electron carrier of methanogenesis (continued).

1.1.4.4. *Overview of the different methanogenic pathways*

All pathways of methanogenesis have in common that methylated CH_3-S-CoM is formed and finally reductively cleaved by the CH_3-S-CoM reductase (MCR) in a coenzyme B (HS-CoB) dependent reaction. This reaction results in the formation of CH_4 and the heterodisulfide CoM-S-S-CoB. The heterodisulfide then acts as the terminal electron acceptor of an electron transport chain which is coupled to the build-up of a sodium and proton gradient. This gradient fuels transmembrane ATPases to form ATP from ADP and P_i. The main differences between the individual methanogenic pathways arise from the way of forming CH_3-S-CoM, which is in all pathways mediated by CH_3Cob(III). Furthermore, the electron transport mechanisms differ which lead to the terminal cleavage of the heterodisulfide formed in the CH_4 releasing step (Deppenmeier 2002). A schematic overview of the three main methanogenic pathways is given in Fig. 1.7.

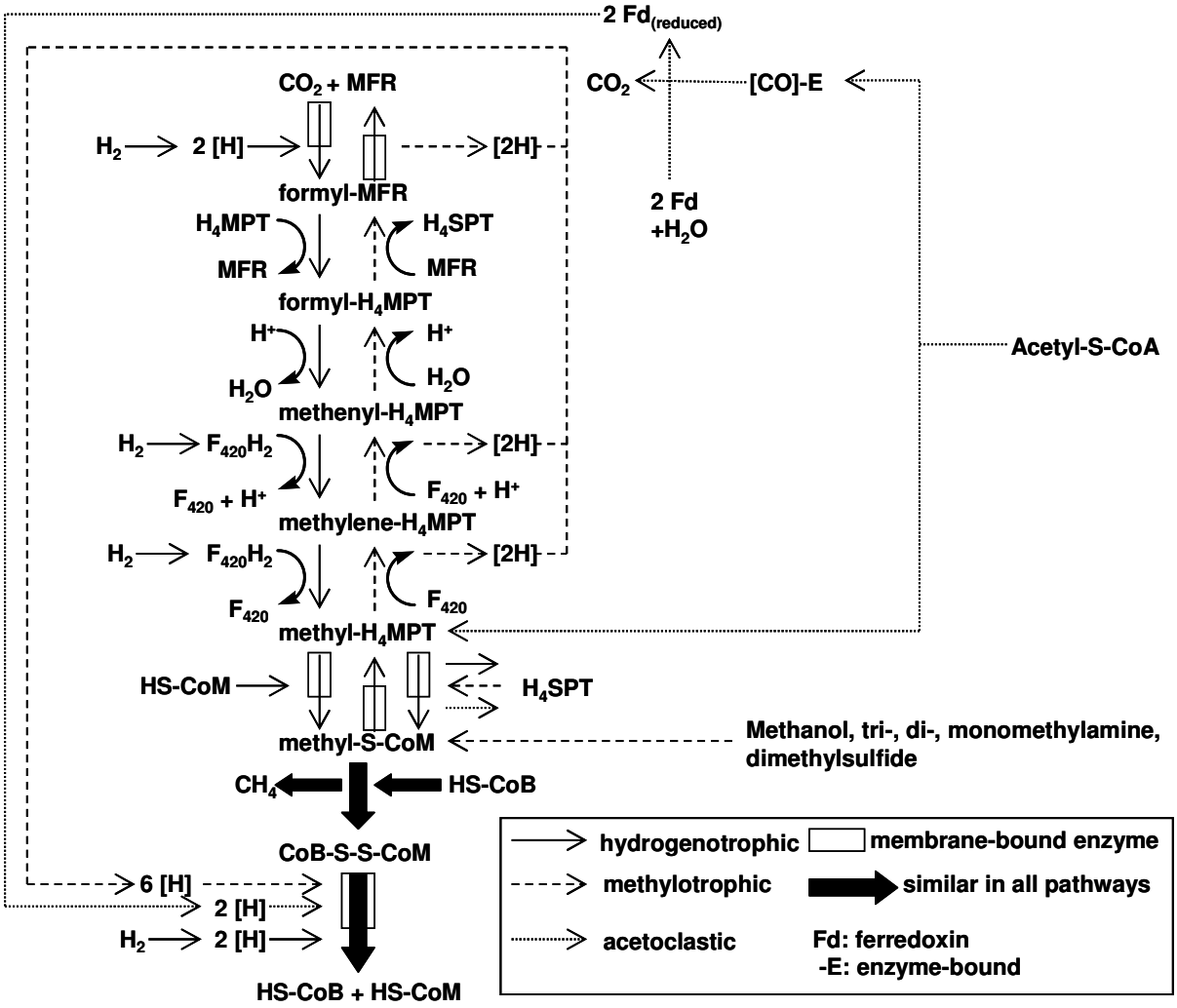

Fig. 1.7: Schematic overview of the three different pathways of methanogenesis.

The hydrogenotrophic methanogenesis starts with the nucleophilic attack of uncharged methanofuran (MFR) upon CO_2, followed by a reduction step catalyzed by the membrane associated formyl-MFR dehydrogenase. The electrons for this first reduction step are provided by H_2, activated by a hydrogenase and mediated via ferredoxin. The first stable product is formyl-methanofuran (CHO-MFR). The formyl group is then transferred to tetrahydromethanopterin (H_4MPT) or its derivative tetrahydrosarcinapterin (H_4SPT) in the case of *Methanosarcinales*. CHO-H_4MPT is then stepwise reduced over methenyl-H_4MPT (CH-H_4MPT) and methylene-H_4MPT (CH_2-H_4MPT) to methyl-H_4MPT (CH_3-H_4MPT). The electrons are provided by reduced cofactor H_2F_{420}. F_{420} itself is reduced by F_{420}-reducing hydrogenases. The methyl group is then transferred via a Cob(I) harboring enzyme complex (methyl-H_4MPT:HS-CoM methyltransferase) from CH_3-H_4MPT to HS-CoM, forming CH_3-S-CoM. The methyl-H_4MPT:HS-CoM methyltransferase couples this exergonic

reaction to sodium extrusion, resulting in an electrochemical sodium gradient. The electrons for the terminal reductive cleavage of the heterodisulfide CoM-S-S-CoB are provided by H_2 via an electron transport system designated H_2:heterodisulfide oxidoreductase.

In the methylotrophic branch of methanogenesis, methylated C_1 compounds like CH_3OH, $(CH_3)_3N$, $(CH_3)_2NH$, CH_3NH_2 or methylthiols like $(CH_3)_2S$ are demethylated. The methyl group is transferred via a soluble methyltransferase system to HS-CoM, forming CH_3-S-CoM. Three out of four methyl groups bound to $^-$S-CoM are reductively cleaved like in the case of hydrogenotrophic methanogenesis. One methyl group out of four is oxidized to CO_2 in the reversed path of hydrogenotrophic methanogenesis to form reduced H_2F_{420}. The electrons of H_2F_{420} are used for the reductive cleavage of CoM-S-S-CoB via the H_2F_{420}:heterodisulfide oxidoreductase. The electron transport is mediated by methanophenazine and again coupled to proton extrusion.

The soluble methyltransferase system involved in the formation of CH_3-S-CoM comprises three polypeptides: A substrate specific enzyme, a corrinoid (Cob(I)) harboring protein, and a HS-CoM methylating enzyme. In terms of methylotrophic methanogenesis from CH_3OH these polypeptides are designated MtaB, MtaC and MtaA, respectively (Fig. 1.8).

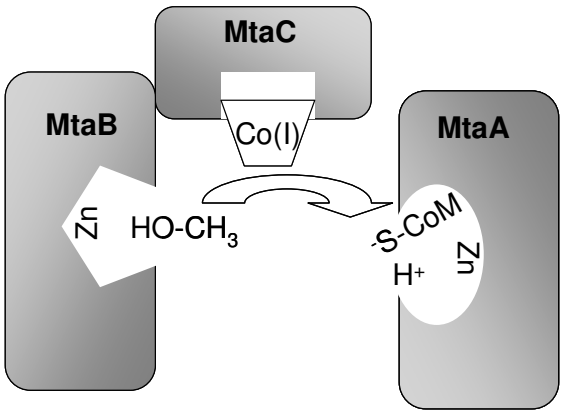

Fig. 1.8: Schematic of the soluble methyltransferase system involved in methanogenesis from methanol.

The corrinoid-binding MtaC from *M. barkeri* shows 35% homology to the methionine synthethase MetH from *E. coli* (Matthews et al. 2008). The three-dimensional structure of the MtaBC dimer complex was solved in 2006 (Hagemeier et al. 2006). There are further three sets of substrate cleaving and corrinoid binding enzymes for

the methylated amines which are highly specific for their substrate. Also a second HS-CoM methylating enzyme designated MtbA is involved. In the case of methanogenesis from methylthiols, only the two polypeptides MtsA and MtsB are required. The latter one possesses a corrinoid-binding motif and is hence harboring a Cob(I) prosthetic group. MtsA, instead, catalyzes both, the demethylation of the methylated thiol substrate and the CH_3Cob(III)-dependent HS-CoM methylation.
Methanogenesis from acetate is considered to be the most important pathway of methanogenesis in terms of total global biogenic methane production. However, the capability to use acetate as substrate for methanogenesis is restricted on two genera, *Methanosarcina* and *Methanosaeta*. Initially, the carbonyl group of acetate is activated by an acetate kinase in an ATP-dependent reaction, forming acetyl-phosphate. Acetyl-phosphate is then transferred to coenzyme A (CoA) forming acetyl-CoA. This step is catalyzed by a phosphotransacetylase (Pta). The key enzyme of the acetoclastic methanogenesis is the carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS). This enzyme consists of five subunits including one corrinoid (Cob(I)) binding subunit (Grahame and DeMoll 1996; Gencic and Grahame 2008). This enzyme cleaves the C-C and C-S bonds of acetyl-CoA, forming a methyl group that is transferred under involvement of Cob(I) to H_4MPT, and a protein-bound carbonyl that is oxidized to CO_2. The resulting electrons from the CO oxidations are used to form reduced ferredoxin. The CH_3-H_4MPT is demethylated by the methyl-H_4MPT:HS-CoM methyltransferase, forming CH_3-S-CoM like in the case of hydrogenotrophic and methylotrophic methanogenesis. The electrons used for the terminal reduction of the heterodisulfide are provided by the reduced ferredoxin formed upon the oxidation of CO to CO_2.

1.1.5. *Methanosarcina mazei*: The model organism

Methanosarcina mazei belongs to the order *Methanosarcinales* and can utilize methylated C1 compounds, methylated thiols, acetate as well as CO_2 and H_2 for methanogenesis. The strain *M. mazei* Gö1 (Fig. 1.9), used in this thesis, was initially isolated from a sewage plant in Göttingen, Germany (Jussofie et al. 1986). *M. mazei* is irregular spherically shaped and forms cell aggregates (sarcines). This methanoarchaeum was a valuable model organism for the elucidation of the electron transport chain in methanoarchaea, entailing further research. Hence, *M. mazei* is one of the best studied methanoarchaea to date (Spanheimer and Muller 2008). The

genome sequence was published in 2002, revealing a length of 4.1 Mb with about 75% coding regions (Deppenmeier et al. 2002). Noticeable, sequence homology analyses revealed a high number of *orf*s with closest homology to bacterial *orf*s (31%), suggesting numerous lateral gene transfer events. *M. mazei* is closely related to *M. acetivorans* and *M. barkeri* (Maeder et al. 2006).

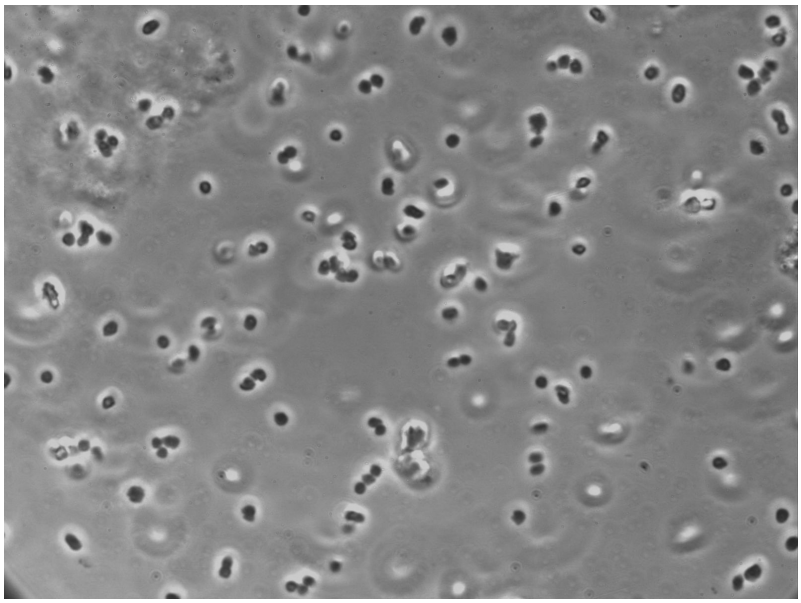

Fig. 1.9: Phase contrast microscopic picture of a *M. mazei* culture (1250-times magnified).

In 2005, the first whole genome microarray analyses of *M. mazei* was published, comparing differential gene expressions when grown on methanol compared to growth on acetate (Hovey et al. 2005). The used microarray chip covers 97% of all predicted *orf*s. The same year, a genetic method to construct *M. mazei* knock-out mutants was established, basing on a liposome-mediated transformation protocol (Ehlers et al. 2005). Further analyses of transcriptional response to various environmental conditions were performed in the following years.

Another interesting feature of *M. mazei* is its capability to convert numerous metal(loid)s into volatile permethylated derivatives (Meyer et al. 2008). The combination of high versatility in metal(loid) methylation and the established protocol for performing genome wide transcriptome analyses making *M. mazei* the perfect model organism to study the distinct capability of methanoarchaea to methylate metal(loid)s.

1.2. Aims of this thesis

This work will investigate whether the remarkable versatility of methanoarchaea to convert inorganic metal(loid) compounds into volatile methyl and hydride derivatives is directly linked to methanogenesis. Therefore, analyses with cell-free crude extracts of *Methanosarcina mazei* will be performed as well as transcriptome analyses of *M. mazei* cultures exposed to elevated bismuth and arsenic concentrations. The derived data shall answer the question whether methylation of various metal(loid)s is conducted by side reactions of the central energy metabolism, methanogenesis, or by more specific pathways.

Furthermore, an important $CH_3Cob(III)$ depending methyltransferase from methylotrophic methanogenesis, MtaA, will be tested for its ability to mediate formation of metal(loid) methyl and hydride derivatives *in vitro*. These analyses may contribute to the understanding of the mechanistic of $CH_3Cob(III)$ dependent metal(loid) methylation and hydride generation in methanoarchaea.

Besides investigations concerning metal(loid) methylation and hydride generation in methanoarchaea, this study shall also deal with possible cell damaging aspects of elevated metal(loid) concentrations as exemplified on bismuth and arsenic.

2. Materials and methods

2.1. Chemicals, enzymes, kits and consumables

[α-^{32}P]-CTP	Hartman Analytics; Braunschweig, Germany
Agarose, peqGold Universal	peQLAB Biotechnologie GMBH; Erlangen, Germany
Acrylamide, N,N-methylenebisacrylamide	SERVA GmbH; Heidelberg, Germany
Calf intestinal (alkaline) phosphatase (CIP)	Promega GmbH; Mannheim; Germany
Antibiotics (ampicilin and kanamycin)	Sigma-Aldrich; Taufkirchen, Germany
Bradford Reagent	BioRad; München, Germany
Cy5-/Cy3-dCTP	Amersham Bioscience; Freiburg, Germany
CyScribe First-Strand cDNA Labeling Kit	Amersham Bioscience; Freiburg, Germany
CyScribe GFX Purification Kit	Amersham Bioscience; Freiburg, Germany
Bovine serum albumin (BSA)	Sigma-Aldrich; Taufkirchen, Germany
Comassie-Brilliant-Blue R and G	SERVA GmbH; Heidelberg, Germany
Dialysis tubes	Roth GmbH; Karlsruhe, Germany
Diethyl pyrocarbonat (DEPC)	AppliChem GmbH; Darmstadt, Germany
DNA-ladder (1 kb), GeneRulerTM, ready-to-use	Fermentas Life Sciences; St. Leon-Rot, Germany
DNA-Polymerase, (*Thermus aquaticus*)	Fermentas Life Sciences; St. Leon-Rot, Germany
DNA-Polymerase, (*Pyrococcus furiosus*)	Fermentas Life Sciences; St. Leon-Rot, Germany
DNase I, on-column	Qiagen; Hilden, Germany
DNeasy Tissue Kit	Qiagen; Hilden, Germany

dNTP Mix	Fermentas Life Sciences; St. Leon-Rot, Germany
GeneJET™ Plasmid Miniprep Kit	Fermentas Life Sciences; St. Leon-Rot, Germany
Isopropyl-ß-thiogalactopyranosid (IPTG)	Gerbu Handelsgesell.; Gaiberg, Germany
Lucidea Universal ScoreCard	Amersham Bioscience; Freiburg, Germany
Microarray Hybridization Solution, Version 2.0	Amersham Bioscience; Freiburg, Germany
Ni-NTA agarose	Qiagen; Hilden, Germany
Roti®-Nylon plus, nylon membrane	Roth GmbH; Karlsruhe, Germany
Oligonucleotides	Thermo Fisher Scientific Inc.; Waltham, USA
Restriction endonucleases	Fermentas Life Sciences; St. Leon-Rot, Germany
unstained Protein Ladder, (10-200 kDa)	Fermentas Life Sciences; St. Leon-Rot, Germany
QIAquick Gel Extraction Kit	Qiagen; Hilden, Germany
QIAquick PCR Purification Kit	Qiagen; Hilden, Germany
RNA ladder, high range	Fermentas Life Sciences; St. Leon-Rot, Germany
RNase A	Merck Biosciences; Darmstadt, Germany
RNase-ExitusPlus™	AppliChem GmbH; Darmstadt, Germany
RNeasy Midi Kit	Qiagen; Hilden, Germany
Scintillation liquid Rotiszint eco plus (ready-to-use)	Roth GmbH; Karlsruhe, Germany
SDS	SERVA GmbH; Heidelberg, Germany
Sodium tetraethylborate solution	(1% w/w) (Galab; Geesthacht, Germany)
Sodium tetrahydroborate (>99% purity)	(Acros Organics; Geel, Belgium) stabilized by 2% (w/w) KOH-solution)

T4-DNA-ligase	Fermentas Life Sciences; St. Leon-Rot, Germany
T7 *in vitro* transcription kit	Fermentas Life Sciences; St. Leon-Rot, Germany
Type 7* Microarray-Slides	Amersham Bioscience; Freiburg, Germany
ULTRAhyb solution	Applied Biosystems/Ambion; Darmstadt, Germany
Whatman GB 004, 3MM	Schleicher & Schuell GmbH; Dassel, Germany

Organic solvents were derived from J.T. Barker (Deventer, Netherlands). All chemicals not listed above were from AppliChem (Darmstadt, Germany), Fluka (Buchs; St. Gallen, Schweiz), Gerbu (Gaiberg, Germany), Merck (Darmstadt, Germany) or Sigma Aldrich (Taufkirchen, Germany) and were analytical grade unless otherwise indicated. Aqueous solutions were prepared using double deionized water derived from a pure water unit (Milli-Q Biocel A10, Millipore; Molsheim, France). For molecular biological applications, nuclease and nucleic acid-free water purchased from Fermentas (Fermentas Life Sciences; St. Leon-Rot, Germany) was used. Liquid nitrogen as well as gases for ICP-MS and EI-MS analytic were purchased from Air Liquide (Düsseldorf, Germany). Element standard solutions for ICP-MS analytics (for internal standard calibration) were purchased from Kraft (Duisburg, Germany) and Merck (Darmstadt, Germany).

2.2. Instruments

Anaerobic glove box	Vinyl Anaerobic glove box, Typ B (Coy Laboratory Products Inc.; Grass Lake, MI, USA)
Aqua bidest. water system	Milli Q Biocel A10 (Millipore; Schwalbach, Germany)
Agarose gel electrophoreses	Easy-Cast Electrophoresis System (Owl Separation Systems; Portsmouth, USA)
Power supply for gel electrophoreses	Consort E143 (Owl Separation Systems; Portsmouth, USA)
Autoclave	Zirbus LVSA 40/60 (Zirbus; Bad Grund, Germany) H+P Variok-lav 75 T (H+P Variok; Oberschleißheim, Germany)

Benchtop heater	BT3 (Grant Instruments; Cambridge, UK)
Cell disruption	French Press (SLM Aminco Instruments Inc.; distributed by Sopra GmbH; Büttelborn, Germany) Mikrodismembrator S (Sartorius; Göttingen, Germany)
Centrifuges	Hettich Universal centrifuge 32R (Hettich; Tuttlingen, Germany) Biofuge® pico and Biofuge A (Heraeus Holding GmbH; Hanau, Germany) Avanti J-25 (Beckman Coulter GmbH; Krefeld. Germany) Centrifuge 5810R (Eppendorf; Hamburg, Germany)
Centrifugal Filter Devises	Centricon (Millipore; Schwalbach, Germany)
Chemiluminescence detector for gel documentation, including video copy processor	ChemiDoc Gel Documentation System (BioRad Laboratories GmbH; München, Germany) P91W B/W thermal printer (Mitsubishi Electronics America, Inc.; Ratingen, Germany)
Chromatography columns and material	Superdex 200 HiLoad 26/60 column (GE Healthcare; Freiburg, Germany) Q-Sepharose FastFlow (GE Healthcare; Freiburg, Germany) Ni-NTA agarose (Qiagen; Hilden, Germany)
Contamination monitor	Contamat FHT 111M LB 124, CA (USA) (Berthold Technologies GmbH; Bad Wildberg, Germany)
EI-MS	5973 N EI-MS (Agilent Technologies; Waldbronn, Germany)
GC-Systems	semi-automated system as described previously (Diaz-Bone and Hitzke 2008; Wuerfel et al. 2009) 6890 N gas chromatographic system (Agilent Technologies; Waldbronn, Germany) equipped with UNIS 2000 inlet system

	(Joint Analytical Systems; Moers, Germany)
	Home-build GC System
	for bismuth ethylation experiments
	(University of Duisburg-Essen; Essen, Germany)
High pressure liquid chromatography (HPLC)	HPLC System Gold, 126NM Solvent Module, 166NM Detector (Beckman Coulter; Krefeld, Germany)
Hybridization chamber for microarrays	Lucidea SlidePro hybridization chamber (GE Healthcare; Freiburg, Germany)
Hybridization oven	OV3 Mini hybridisation oven (Biometra; Göttingen, Germany)
ICP-MS	Elan 6000 (PerkinElmer; Rodgau, Germany)
	7500a ICP-MS (Agilent; Yokohama, Japan)
Incubator	Präzisionsbrutschrank ICP 600 (Memmert; Schwabach, Germany)
Microscope	Olympus BH-2 RFCA (Olympus; Hamburg, Germany)
pH meter	WTW pH 197 (WTW GmbH; Weilheim, Germany)
Phosphor imager and data processing	Image Reader FLA 5000, V2.1; (Fujifilm; Tokyo, Japan) AIDA software (Fujifilm; Tokyo, Japan)
Photometer	Specord 200 with WinASPECT Spectralanalysis-Software (Analytik Jena AG; Jena, Germany)
	Eppendorf BioPhotometer (Eppendorf; Hamburg, Germany)
	Genesys 20 (Thermo Spectronics; Rochester, NY, USA)
Polyacrylamide gel electrophoresis (SDS-PAGE)	Mingel-Twin (Biometra; Göttingen, Germany)
Scanning of the microarrays and image analyses	GenePix 4100A scanner with GenPix Pro software version 6.0 (Axon Instruments; Union City, USA)
Scintillation Counter	Liquid Scintillation Counter Wallac 1409 (PerkinElmer, Wallac; Turku, Finnland)
Thermal cycler	MJ Mini and iCycler (BioRad Laboratories GmbH;

	München, Germany)
Vacuum centrifuge	SpeedVac Concentrator
	(Savant Instruments, Inc.;
	Farmingdale, NY, USA)

2.3. Software and online tools

2.3.1. Software

Autoradiogram analyzes	AIDA software
	(Fujifilm; Tokyo, Japan)
Gel documentation	Quantity One
	(BioRad Laboratories GmbH; München, Germany)
Microarray data processing	GenPix Pro software version 6.0
	(Axon Instruments; Union City, USA)
Tabular calculations	Microsoft Excel
and numeric value processing	(Microsoft Corporation; Redmond, USA)
	Origin 5.0 and 8.5
	(OriginLab Corporation; Northampton, MA, USA)
Text processing	MS Word
	(Microsoft Corporation; Redmond, USA)
	WordPad
	(Microsoft Corporation; Redmond, USA)
UV-Vis processing	WinAspect
	(Analytik Jena AG; Jena, Germany)

2.3.2. Online tools

DNA and Protein sequence similarity	BLAST
and homology searches	(http://blast.ncbi.nlm.nih.gov/Blast.cgi)
Literature search	PubMed
	http://www.ncbi.nlm.nih.gov/pubmed/
	ISI Web of Knowledge
	http://apps.isiknowledge.com/
DNA and protein sequence alignments	Clustal W 2.0
	http://www.ebi.ac.uk/Tools/msa/clustalw2/
Primer design	PCR Primer Stats
	http://www.bioinformatics.org/sms2/pcr_primer_stats.html
M. mazei genome information	UCSC Archaeal Genome Browser
	http://archaea.ucsc.edu/cgi-bin/hgGateway?db=methMaze1

2.4. Microorganisms

Methanosarcina mazei Gö1 (DSM 3647) was derived from the “Deutsche Sammlung für Mikroorganismen und Zellkulturen“ (DSMZ; Braunschweig, Germany).
Cloning and expression of recombinant enzymes was done in the following *E. coli* K12 derivatives:

Escherichia coli K12 DH5α strain; DSMZ 6897 (Hanahan 1983)
Escherichia coli BL21(DE3); Novagen (Studier and Moffatt 1986)

2.4.1. Cultivation of microorganisms

Two different microorganisms were used in this thesis: *Methanosarcina mazei* and *Escherichia coli*. *M. mazei* was used for the main research, including analyses on the formation of non-volatile methyl bismuth derivatives, formation of volatile metal(loid) derivatives by cell-free crude extracts and for transcriptome analyses. *E. coli* was used for heterologous overexpression of *M. mazei* methyltransferases.
The cultivation of these organisms was adapted to the respective application as described in the following sections.

2.4.1.1. *Cultivation of* M. mazei *for experiments with growing cultures*
Cultivation of *M. mazei* was performed in modified DSMZ-120 complex medium. Composition of the growth medium as well as the vitamin solution and trace element solution are given in Tab. 2.1, Tab. 2.2 and Tab. 2.3.

Tab. 2.1: Composition of the used *M. mazei* complex medium.

compound	***amount [g] resp. [ml]****
K_2HPO_4	*0.348*
KH_2PO_4	*0.227*
NH_4Cl	*0.500*
$MgSO_4$ x 7 H_2O	*0.500*
$CaCl_2$	*0.250*
$C_2H_3NaO_2$ x 3 H_2O	*1.500*
$NaCl_2$	*2.250*
yeast extract	*2.000*
casitone	*2.000*
$FeSO_4$ x 7 H_2O	*0.002*
resarzurin solution (0,1%)	*1.0**
vitamin solution	*10.0**
trace element solution SL6	*3.0**
$NaHCO_3$	*0.850*
H_2O	*ad 1000.0**

Tab. 2.2: Vitamin solution (Wolin et al. 1964), modified.

compound	***amount [mg] resp. [ml]****
biotin	*2.0*
folic acid	*2.0*
pyridoxine hydrochloride	*10.0*
thiamine hydrochloride	*15.0*
riboflavin	*5.0*
nicotinic acid	*5.0*
D-Ca-pantothenic	*5.0*
vitamin B_{12}	*10.0*
p-amino benzoic acid	*5.0*
lipoic acid	*1.0*
H_2O	*ad 1000.0**

Tab. 2.3: Trace element solution SL6 (10x) (Pfennig and Lippert 1966).

compound	***amount [mg] resp. [ml]****
$ZnSO_4$	*100.0*
MnCl x 4 H_2O	*30.0*
H_3BO_3	*300.0*
$CoCl_2$ x 6 H_2O	*200.0*
$CuCl_2$ x 2 H_2O	*10.0*
Na_2MoO_4 x 2 H_2O	*30.0*
H_2O	*ad 1000.0**

The medium was purged for 30 min with N_2/CO_2 (80%/20%) to remove oxygen. The pH was adjusted to 6.8 by addition of the appropriate volume of a 10 M NaOH solution. Glass vials (120 mL) were filled with 50 mL complex medium, gas-tight sealed with butyl rubber stoppers and gently pressurized with N_2/CO_2 (80%/20%) (500–800 hPa). After heat sterilizing at 121 °C for 20 min, media were supplemented with methanol (250 mM) as main carbon source and L-cysteine (0.3 g L^{-1}) to reduce traces of oxygen. Na_2S was not used as reductant to avoid formation of insoluble metal(loid)-sulfides. To reduce the risk of contamination with other microorganisms, ampicilin and kanamycin (100 µg mL^{-1} each) were added. Finally, the media were inoculated with an appropriate *M. mazei* pre-culture (1%) derived from stock. The cultures were propagated for maximal 7 days in darkness at 37 °C without shaking. Fresh media were inoculated once a week to maintain the culture stock. Growth was monitored by determining the turbidity of the cultures photometrically at λ = 660 nm.

2.4.1.2. *Cultivation of* M. mazei *for preparation of total RNA*

For transcriptome analyzes of bismuth or arsenic exposed *M. mazei*, cultures were grown in 300 mL complex medium in 1 L bottles (air-tight sealed with butyl rubber stopper) for 3 days without metal(loid)s until reaching the exponential growth phase. Then, the 300 mL cultures were divided into 4 times 50 mL aliquots (filled into 120 mL glass vials) in an anaerobic glove box(H_2/N_2 (2%/98%)). Two out of four 50 mL aliquots were supplemented with $Bi(NO_3)_3$, $As(OH)_3$ or KH_2AsO_4, respectively (concentrations are indicated in the result section) and the remaining two 50 mL aliquots prepared from the same 300 mL pre-culture with either KNO_3 as negative control for $Bi(NO_3)_3$ experiments or without amendment (negative control for analyses of transcriptional response towards arsenic). The 120 mL glass vials were gas-tight sealed with butyl rubber stoppers in the anaerobic glove box and subsequently gently pressurized with N_2/CO_2 (80%/20%). The cultures were propagated at 37 °C for additional 48 h prior to RNA isolation (section 2.6.2).

2.4.1.3. *Preparation of* M. mazei *crude extract*

Crude extracts of *M. mazei* were prepared from 500 mL cultures grown for 5–6 days as described above (section 2.4.1.1). No metal(loid) compounds were added to these cultures in order to study metal(loid) methylation and hydride generation activity of non-induced cells. Cells were harvest by centrifugation at 6000 x g for 30 min at 4 °C. Supernatant was discarded and the remaining cell pellets (200 mg) instantly frozen in

a -80 °C freezer. The cell pellets were resuspended in 200 µL 50 mM Tris/HCl (pH 7.0), frozen in liquid nitrogen and disrupted by a Mikro-Dismembrator S laboratory ball mill (Sartorius; Göttingen, Germany) within 10 min at a shaking frequency of 1800 rpm. Cell debris as well as insoluble cell fractions were removed by vigorous centrifugation at 13000 x g for 30 min at 4 °C. The supernatant was collected and protein content determined (refer to section 2.7.3).

2.4.1.4. E. coli *standard cultivation*

E. coli was aerobically propagated in heat sterilized lysogeny broth (LB) (10 g tryptone, 5 g yeast extract, 10 g NaCl solved in 1 L H_2O, pH 7.2) under shaking (180-200 rpm) at 37 °C. The LB media was inoculated with 0.1% of an appropriate *E. coli* pre-culture. Recombinant *E. coli* strains which acquired resistance against ampicillin or kanamycin via a pET-vector plasmid were grown in the presence of the respective antibiotic. Growth was monitored by measuring the turbidity of the cultures photometrically at λ = 600 nm.

2.4.1.5. *Preparation of competent* E. coli *cells*

Competent cells of *E. coli* were chemically prepared by using high ionic strength buffer. Therefore, 100 mL LB medium was inoculated with 1 mL of an appropriate overnight culture of *E. coli* and incubated at 37 °C in a rotary shaker until an OD_{600} of 0.5-0.6 was reached. The culture was then chilled on ice for 5 min prior to centrifugation (6 min at 6000 x g, 4 °C). The pellet was resuspended in 40 mL sterile buffer A (30 mM K-Acetate, 10 mM $CaCl_2$, 50 mM $MnCl_2$, 100 mM KCl and 15% (v/v) glycerol) and incubated on ice for 5 min. After a second centrifugation step (6 min at 6000 x g, 4 °C), the pellet was resuspended in 4 mL sterile buffer B (10 mM MOPS, 75 mM $CaCl_2$, 10 mM KCl and 15% (v/v) glycerol) and stored in 200 µl aliquots at -80 °C.

2.5. Molecular working with DNA

2.5.1. Preparation of genomic DNA from *M. mazei*

Genomic DNA was isolated from growing *M. mazei* cultures using the DNeasy kit (Qiagen; Hilden, Germany). 1-2 mL cell suspension were withdrawn from *M. mazei* pure cultures, cells were briefly centrifuged, the supernatant discarded and genomic

DNA isolated according to the manufacturer's protocol for isolation of genomic DNA from gram-negative bacteria.

2.5.2. Isolation of plasmid DNA from *E. coli*

Plasmid DNA from *E. coli* was isolated by alkaline lyses and subsequent isopropyl precipitation in accordance to the slightly modified protocol given by Birnboim and Doly (Birnboim and Doly 1979). 2 mL of an *E. coli* culture, propagated for about 16 h, was centrifuged for 5 min at 6000 x g. The supernatant was discarded and the pellet resuspended in 300 µL buffer 1 (50 mM Tris/HCl, 10 mM EDTA pH 8, 100 µg mL^{-1} RNAse A) followed by addition of 300 µL buffer 2 (200 mM NaOH, 1% SDS) and gently mixing by inverting the reaction tube for several times. Cells were then lysed within 5 min at room temperature followed by neutralization and precipitation of genomic DNA by adding 300 µL ice chilled buffer 3 (3 M potassium acetate pH 4.8) and concomitant incubation on ice for 20 min. Precipitates, including genomic DNA, denaturized proteins and membrane structures, were separated from the plasmid DNA harboring soluble fraction by centrifugation at 14000 x g for 15 min at 4 °C. The supernatant was withdrawn, plasmid DNA precipitated by adding 0.7 volumes of isopropyl followed by incubation for 10 min at room temperature. Precipitated plasmid DNA was washed with 70% ethanol, dried and finally solved in water. For preparation of plasmid DNA with higher purity e.g. for automated sequencing, plasmid DNA was isolated with the GeneJET™ Plasmid Miniprep Kit (Fermentas; St. Leon-Rot, Germany) according to the manufacturer's instructions.

2.5.3. Amplification of genomic DNA

The PCR technique enables the exponential enzymatic amplification of a specific DNA sequence determined by two specific oligonucleotide primers, one hybridizing to the 5'-end of the coding and the other one to the 5'-end of the non-coding strand, respectively, thereby flanking the sequence region that has to be amplified (Mullis et al. 1986; Saiki et al. 1988).

The elongation of the primers is catalyzed by heat-stable DNA polymerases like the so-called Taq-polymerase of *Thermus aquaticus* or the Pfu-polymerase of *Pyrococcus furiosus*. The latter possesses an additional 3'-5' exonuclease activity ("proofreading-activity"). The PCR reaction mixtures (25-50 µL) contained 50-100 ng

template dsDNA, 2.5 µM of each primer (forward and reverse), 1.5 mM $MgCl_2$ and 200 µM dNTPs unless otherwise indicated. In general, 1 U of DNA polymerase (Taq or Pfu polymerase; One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 70°C.) with the accordant reaction buffer was used per 25 µL reaction mixture. The PCR reaction was performed using a MJ Mini Gradient Thermal Cycler (Bio-Rad Laboratories GmbH; München, Germany). A standard PCR reaction includes the following main steps:

Denaturation: dsDNA denaturation to form ssDNA at 95 °C for 2 min;

Primer annealing: Hybridisation of the oligonucleotide-primers to their complementary DNA sequence at primer specific annealing temperatures;

Primer extension: DNA polymerase catalyzed elongation of the primers in 5'-3' direction at 72 °C, thereby synthesizing a complementary copy of the DNA stand;

The DNA target sequence is exponentially amplified by repeating the denaturation, primer annealing and elongation steps for 25-30 times.
Each primer possesses a specific annealing temperature, which depends on the length and the base composition of the oligonucleotide. The annealing temperature is about 3 °C below the melting temperature (T_m in °C). The approximate melting temperature (T_m) for primers shorter than 20 nucleotides was calculated using the following formula (Thein and Wallace 1986):

$$2 \cdot (n_A + n_T) + 2 \cdot (n_G + n_C) = T_m$$

with n_A: number of adenine
n_T: number of thymidine
n_G: number of guanidine
n_C: number of cytosine

The calculated melting temperatures were used as reference points to determine the annealing temperature ($T_{annealing}$) empirically.

2.5.4. Agarose gel electrophoresis

The electrophoretic separation of DNA molecules by agarose gel electrophoresis was used to determine the length of DNA molecules (plasmid-DNA and PCR-products), to control progression of restriction enzyme reactions and to separate DNA fragments with specific length from unspecific DNA fragments for cloning experiments (Sambrook et al. 1989).

Agarose solved in TAE buffer (40 mM Tris-acetate, 1 mM EDTA) to yield 1.0% (w/v) was used for electrophoresis. Electrophoresis was performed with TAE-buffer as running buffer at a voltage of 60-100 V depending on the gel size. The DNA samples were mixed with loading dye (6x: 0.2% bromophenol blue, 0.2% xylene cyanol FF, 60% (v/v) glycerol, 60 mM EDTA) and applied into the sample wells. In addition to the DNA samples added, 5 µL of a DNA-ladder containing a mixture of DNA fragments of known size as reference (GeneRulerTM, 1kb DNA ladder (Fermentas Life Sciences; St. Leon-Rot, Germany)) was loaded onto the gel in a separate pocket. After the electrophoresis run, the DNA was visualized by bathing the gel in an ethidium bromide solution (2 mg L^{-1}) for about 30 min prior to detection upon UV light irradiation (λ = 254 nm) using a ChemiDoc Gel Documentation System (Bio-Rad Laboratories GmbH; München, Germany). The sizes of the separated DNA fragments were determined according to their relative positions to DNA fragments of known length from the applied DNA-ladder.

2.5.5. Purification of DNA fragments

For the extraction and purification of DNA fragments from agarose gels or from PCR reaction mixtures, the QIAquick gel extraction kit or the QIAquick PCR purification kit were used, respectively, according to the instructions of the manufacturer (Qiagen; Hilden, Germany).

2.5.6. Plasmids and constructed recombinant vectors

Two *orf*s of *M. mazei*, encoding designated methyltransferases MtaA (MM_0176 and MM_1070), were inserted into pET-15b or pET-24a plasmid vectors (Tab. 2.4), respectively, resulting in the constructs listed in Tab. 2.5. Primers which introduce restriction recognition sites adjacent to the translation start codon at the 5'-end and to the translation stop at the 3'-end were used for the amplification of dsDNA fragments encoding CH_3Cob(III) dependent methyltransferase MtaA (MM_0176 and MM_1070).

Tab. 2.4: Plasmids used in this work.

vector	resistance	application	source of supply
pET-15b	Amp^r	heterologous expression of *M. mazei* proteins in *E. coli*	Novagen, Merck Bioscience
pET-24a	Kan^r	heterologous expression of *M. mazei* proteins in *E. coli*	Novagen, Merck Bioscience

Tab. 2.5: List of constructed recombinant vectors and the according primers used to amplify genes of *M. mazei* by PCR.
The restriction sites of primers for facilitating the insertion of the PCR products into the multiple cloning sites of the used plasmids are underlined. The annealing temperatures are indicated at which specific amplification of target DNA sequence by PCR was detected.

plasmid + insert	primer sets	restriction site	sequence (5' → 3')	$T_{annealing}$
pET15b+MM_0176	MM0176for	*NdeI*	GGAGATTCATATGGGTGATA	40 °C
	MM0176rev	*XhoI*	AACACACTCGAGTAAAAAATG	40 °C
pET24a+MM_0176	MM0176for	*NdeI*	GGAGATTCATATGGGTGATA	40 °C
	MM0176rev	*XhoI*	AACACACTCGAGTAAAAAATG	40 °C
pET15b+MM_1070	MM1070for MK3	*NdeI*	CGCGGAATTCATATGACCGATATGAGCGAATTC	60 °C
	MM1070rev MK3	*XhoI*	AGAGCGGATCCTCGAGTCAGGCGTAGAATTC	60 °C
pET24a+MM_1070	MM1070for MK3	*NdeI*	CGCGGAATTCATATGACCGATATGAGCGAATTC	60 °C
	MM1070rev MK3	*XhoI*	AGAGCGGATCCTCGAGTCAGGCGTAGAATTC	60 °C

Constructs of pET-15b were used for expression of *M. mazei* proteins with an N-terminal hexa-histidine tag (His_6-tag) for rapid purification of the recombinant polypeptide on a Ni-NTA column. For expressions of *M. mazei* proteins without a His_6-tag, PCR products were ligated into pET-24a vectors. Here, the His_6-tag encoding region lays downstream the translation stop codon of the inserted gene.

2.5.7. Quantitative and qualitative analyses of DNA

DNA concentrations were determined by absorption of DNA samples at λ = 260 nm. Absorption of 1 at λ = 260 nm is corresponding to a concentration of 50 µg mL^{-1} (double strand DNA) or 40 µg mL^{-1} (single strand DNA) (Sambrook et al. 1989). The purity of DNA preparation was determined by the ratio of absorbance at λ = 260 nm to 280 nm. A value <1.8 or >2.0 points towards contamination by proteins or phenol.

2.5.8. Enzymatic modification of DNA

To construct the recombinant vector plasmids listed in Tab. 2.5, enzymatic modifications of PCR amplified genes from *M. mazei* and the plasmids listed in Tab. 2.4 were performed, including the following reactions:

1. Site specific restriction of DNA
2. 5'-dephosphorylation of linearized plasmid DNA
3. Ligation of complementary overlapping DNA

The restriction of PCR products and plasmid DNA was carried out with the appropriate restriction endonucleases (*NdeI* and *XhoI* (Fermentas Life Science; St. Leon-Rot, Germany)) in accordant buffers following the instructions of the manufacturer. 2-3 U enzyme per µg DNA were used (one unit is defined as the amount of restriction endonuclease required to digest 1 µg lambda DNA in 1 h at 37 °C). The samples were incubated for 1-2 h at 37 °C. Restriction enzymes, buffer and nucleotides <100 bp were removed by QIAquick PCR Purification Kit (Qiagen; Hilden, Germany) or by QIAquick Gel Extraction Kit (Qiagen; Hilden, Germany) after gel electrophoreses according the manufacturer's instructions.

In order to avoid self-ligation of restricted vector DNA during the ligation reaction, the 5'-end phosphate groups were eliminated by calf intestinal alkaline phosphatase (CIAP; Fermentas Life Science; St. Leon-Rot, Germany) treatment. 0.05 U (one unit is the amount of the enzyme required to dephosphorylate 1 µg of linearized pUC57 DNA 5'-termini in 10 min at 37°C) of CIAP $pmol^{-1}$ DNA were added towards the end of the restriction reaction followed by additional incubation at 37°C for 30 min. The

sample was then electrophoretically separated on an agarose gel and purified as described above.

The insertion of restricted DNA fragments (inserts) into vector DNA was carried out by the T4 DNA ligase (Bankier et al. 1987; Pan et al. 1994). The enzyme catalyzes the ATP-dependent formation of a phosphodiester linkage between the 5'-phosphoryl group and the adjacent 3'-hydroxyl group of duplex DNA in a blunt-ended or cohesive-ended configuration.
Equimolar amounts of restricted, dephosphorylated plasmid DNA and insert DNA were used at a ratio of 1:2 or 1:3 in a total volume of 16 µL and incubated at 45 °C for 5 min to solve secondary structures. 2 µL of 10x reaction buffer (400 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM DTT, 5 mM ATP, pH 7.8) and 2 µL T4 DNA ligase (1 weiss-unit μL^{-1}; Fermentas Life Science; St. Leon-Rot, Germany) were added to the reaction mixture yielding a final volume of 20 µL. The ligation reaction was carried out overnight at 8 °C. The T4 DNA ligase was inactivated by incubation at 65 °C for 10 min. The recombinant vector molecules were stored at -20 °C or directly used for transformation.
Recombinant vector molecules successfully transformed into competent *E. coli* strains were isolated from appropriate colonies and the sequence checked by automated DNA sequencing using pET vector primers (Tab. 2.6) at a commercial sequencing facility (AGOWA genomics; Berlin, Germany).

Tab. 2.6: Sequences of pET vector primers (Novagen, Merck Bioscience; Schwalbach, Germany).

primer	sequence (5' → 3')
T7 Promoter Primer	TAATACGACTCACTATAGGG
T7 Terminator Primer	GCTAGTTATTGCTCAGCGG

2.5.9. Transformation of competent *E. coli* cells

For transformation of competent *E. coli* cells (section 2.4.1.5) with plasmid DNA (section 2.5.6), 20 µL of purified circular plasmid DNA was gently mixed with 200 µL of competent *E. coli* cells and incubated on ice for 1 h. After 1 h, the cells were subjected to a heat shock at 42 °C for 45 s and subsequently stored on ice for additional 5 min to promote the transformation. 800 µL of LB medium was added to

the sample which was subsequently incubated at 37 °C for about 1 h in a rotary shaker. 100 µL of transformed cells were plated on LB agar plates containing the appropriate antibiotics. After incubation at 37 °C overnight, colonies were screened for positive clones, carrying the recombinant plasmid, using colony screening PCR (refer to section 2.5.10) or restriction digestion of isolated plasmid DNA (section 2.5.8) with subsequent analyses of DNA fragment sizes by agarose gel electrophoresis (see section 2.5.4).

2.5.10. Colony screening PCR

Colonies grown on a LB agar plate containing appropriate antibiotics to select for acquired antibiotic resistance were gently withdrawn with a pipette tip, spread on a marked area of a new LB agar plate and remaining cells transferred into 50 µL buffer containing 50 mM Tris/HCl pH 7. The resuspended cells were lysed by boiling at 95 °C for 5 min. The lysate was quickly centrifuged and 5 µL of the supernatant used as DNA template in a standard PCR, using specific primers for amplification of DNA inserted into the multicloning site of pET-15b or pET-24a vectors (Tab. 2.5).

2.6. Biomolecular techniques: Working with RNA

2.6.1. Treatment of solutions, glassware and equipment

Working with RNA requires special measures to create and maintain an RNase-free environment. Only solutions, plasticware and enzymes that were certified RNase-free by the manufacturer were used. Self-made buffers and solutions were mixed with 0.1% (v/v) diethyl pyrocarbonate (DEPC) to inactivate RNases. The treated solutions were incubated at RT in a fume hood overnight and then autoclaved to remove traces of DEPC. Glassware was heat sterilized by incubation at 200 °C for at least 2 h prior to use. Non-disposable plasticware was treated with RNase Exitus Plus™ (AppliChem GmbH; Darmstadt, Germany). In order to keep solutions, reagents and consumables such as pipette tips RNase-free, they were stored at a separate place and used only for RNA work.

2.6.2. Isolation of total RNA from *M. mazei*

To isolate total RNA from *M. mazei* cultivated as described under section 2.4.1.2, cultures were transferred from glass vials into centrifugation bottles in an anaerobic glove box. The centrifugation bottles were tightly closed with the appropriate lid, chilled on ice for 10 min and subsequently centrifuged at 6000 x g for 30 min at 4 °C. The supernatant was discarded and total RNA immediately isolated. Therefore, cell pellets were resuspended in 200 µL 50 mM Tris/HCl (pH 7.0), frozen in liquid nitrogen and disrupted by a Mikro-Dismembrator S laboratory ball mill (Satorius; Göttingen, Germany) within 3 min of shaking at a shaking frequency of 1600 rpm. Total RNA was subsequently isolated from cell debris by using the RNeasy Midi Kit (Qiagen) according to the manufactures manual. DNA was digested on-column by using RNase-free DNase I (Qiagen).

2.6.3. Quantitative and qualitative analysis of RNA

The concentration of extracted RNA was determined photometrically at λ = 260 nm. Absorption value of 1 at λ = 260 nm corresponds to 40 µg RNA mL^{-1} (Sambrook et al. 1989). The purity of RNA preparation was determined by the ratio of absorbance at λ = 260 nm to 280 nm. A value <1.8 or >2.0 points towards contamination by proteins or phenol.
The integrity of purified RNA was checked by denaturing formaldehyde agarose gel electrophoresis (refer to section 2.6.4.1) and ethidium bromide staining as well as northern blotting and methylene blue staining (see section 2.6.4).

2.6.4. Northern blot analyses

Northern blot analyses were performed to quantify mRNA levels of certain genes in *M. mazei* in response to elevated bismuth or arsenic concentrations and to validate the microarray experiments performed in this thesis.

2.6.4.1. *Denaturing agarose gel electrophoresis of RNA*

Electrophoretic separation of RNA was achieved under denaturing conditions in agarose-MOPS/formaldehyde gels (Staynov et al. 1972). For 1.2% (w/v) MOPS/formaldehyde gels, 1.2 g agarose was added to 73.8 ml DEPC treated water

and 10 mL 10x MOPS buffer (10x: 200 mM morpholino propane sulfonic acid (MOPS), 50 mM sodium acetate, 10 mM EDTA, pH 7.0) and microwaved for 2 min. After cooling down to approx. 60 °C, 16.2 mL of 37% (v/v) formaldehyde was added. The agarose-formaldehyde gel was poured into the gel tray and a comb (12 wells) was placed into the tray slot. The solidified gel was placed in the electrophoresis chamber and completely covered with 1x MOPS buffer. The RNA samples and the RNA size marker (RiboRuler™ High Range RNA Ladder, Fermentas) were mixed with 1 volumes of 2x RNA Loading Dye, shipped along with RNA size marker (Fermentas), incubated for 10 min at 65 °C and finally chilled on ice. 2.5-7.5 µg RNA and 2 µL RNA marker were loaded onto the gel as recommended by the manufacturer. Electrophoresis was performed at voltage of 75–100 V for 2–2.5 h.

2.6.4.2. *Capillary transfer of RNA to a nylon membrane (northern blot)*

The electrophoretically separated RNA was transferred from the agarose-MOPS/formaldehyde to a positively charged Roti®-Nylon plus transfermembrane (Roth GmbH; Karlsruhe, Germany) by capillary transfer. Therefore, the agarose-MOPS/formaldehyde was first equilibrated in 20x SSC buffer (3 M sodium chloride, 0.3 M sodium citrate, pH 7) two times for 15 min at RT. The membrane was wetted for 1 min with DEPC treated water and then soaked together with three Whatman filter papers (7 x 8.5 cm) in 20x SSC buffer. The blot, consisting of the agarose-MOPS/formaldehyde, wetted membrane and Whatman filter papers, was assembled as shown in Fig. 2.1. The transfer was performed overnight at 4°C. The blot was quickly washed with DEPC treated H_2O to remove salt from the membrane. RNA was UV-crosslinked by irradiating the blot with UV light (λ = 254 nm) for 3 min each side. Methylene staining was performed to visualize the immobilized RNA. The blot was swayed for at least 1 min in 50 mL staining solution (50 mg methylene blue, 6.6 ml 3 M sodium acetate pH 5.2, 1 mL acetic acid (100%) solved in DEPC treated H_2O). Destaining was achieved by washing the membrane 3-4 times with DEPC treated water.

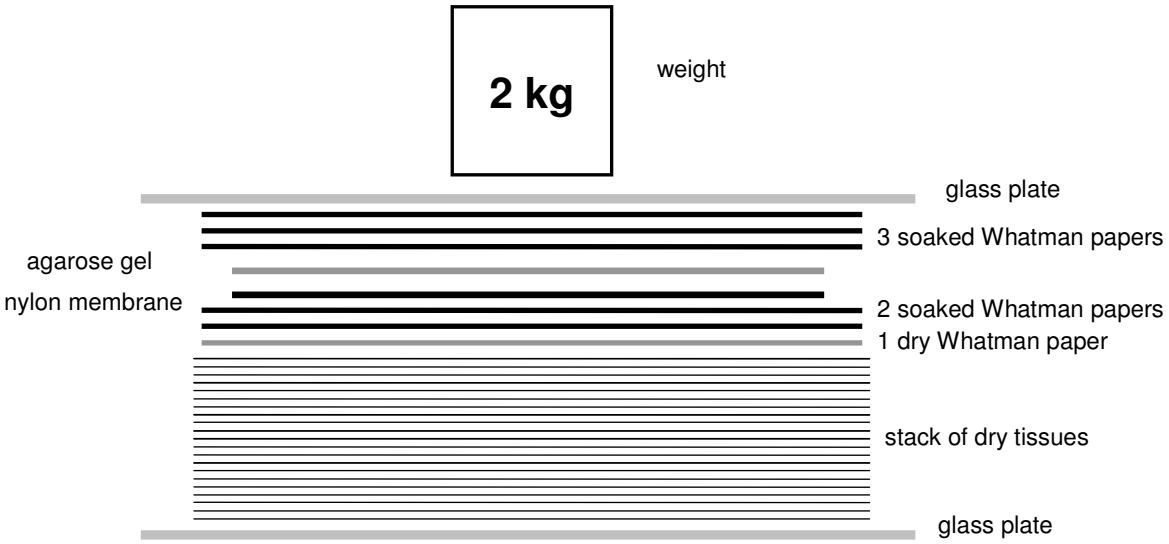

Fig. 2.1: Schematic blot assembly for a capillary transfer of RNA from an agarose gel to a positively charged nylon membrane (northern blot).

The distance between the marker of known size (RiboRuler™ High Range RNA Ladder, Fermentas) and the upper edge of the blot was determined by a ruler to calculate the size of RNA bands after visualizing by hybridization with specific [α-^{32}P]-labeled antisense RNA probes according to their migration.

2.6.4.3. *Hybridization of immobilized RNA with radioactively labeled RNA probes*

After total RNA derived from *M. mazei* cultures was transferred to a positively charged nylon membrane and UV-crosslinked, the RNA was hybridized with radiolabelled antisense RNA probes designed to hybridize specifically with five selected *orf*s (MM_1070, *mtaA*; MM_2242, *arsR*; MM_2243, *arsM*; MM_2962, *mtbB*; MM_3188, *glnA*2) and with the 16S ribosomal RNA. The 16S ribosomal RNA was used as an internal standard. Probe generation was carried out as described in section 2.6.4.4.

2.6.4.4. *Generation of [α-^{32}P]-labeled antisense RNA probes by* in vitro *transcription*

PCR products representing about 500 bp of five selected genes and the 16S rRNA of *M. mazei* were generated. The PCR products served as templates for generating [α-^{32}P]-labeled antisense RNA probes by *in vitro* transcription. For the PCR (100 µL reaction volume), *Taq* polymerase and sequence-specific forward and a modified reverse primers or *vice versa* were used (Tab. 2.7), depending on the orientation of the gene in the genome (+ or - strand). The modified primers constructed comprise

the T7 binding site (5'-TAATACGACTCACTATAGGG-3') and additional six nucleotides (5'-GGGCCC-3') for T7 polymerase binding. The PCR products were purified after gel electrophoresis, quantified photometrically and finally used as templates for probe generation and labeling via *in vitro* transcription.

Tab. 2.7: Sequence of primers used to generate template DNA for T7 *in vitro* transcription. The six nucleotides required for polymerase binding are in italics, the T7 binding site is underlined. Annealing temperatures used in the PCR to generate DNA templates are indicated as well.

primer	sequence (5' → 3')	$T_{annealing}$
MM 16S for T7	*GGGCCC*TAATACGACTCACTATAGGGGTCTTCAGCTTGGCCTACAT	56 °C
MM 16S rev	TCTGAGACATGAATCCAGGC	56 °C
MM1070for MK3	CGCGGAATTCATATGACCGATATGAGCGAATTC	56 °C
MM1070rev T7	*GGGCCC*TAATACGACTCACTATAGGGCATTTCATGAAGGATTTGACA	56 °C
MM2242for	CCATATAATCATGCTTGATAGT	40 °C
MM2242rev T7	*GGGCCC*TAATACGACTCACTATAGGGTCAACCCGGTTTTCAGTACT	40 °C
MM2243for	CGCGGAATTCATATGGATGCGAATGAAA	46 °C
MM2243rev T7	*GGGCCC*TAATACGACTCACTATAGGGAGAACACGGAACG	46 °C
MM2962for	ATGGCAACCGAATATGCTTTA	51 °C
MM2962rev T7	*GGGCCC*TAATACGACTCACTATAGGGTAAGGTGCGCGGCTGCAT	51 °C
MM3188for	CCAGCAAGTGAATTCACCAAAG	60 °C
MM3188rev T7	*GGGCCC*TAATACGACTCACTATAGGGGGCTTCGGCAATTGCGAGCA	60 °C

In vitro transcription

In vitro transcription was performed by using the T7 transcription kit (Fermentas Life Sciences; St. Leon-Rot, Germany) according the manufacturer's instructions. A standard reaction assay (20 µL) contained:

100-200 ng purified PCR product,
0.5 mM rATP, rGTP, rUTP,
12 µM rCTP
50 µCi [α-^{32}P]-rCTP (400 Ci mmol^{-1}), 6.25 µM final concentration
4 µl Reaction buffer (5 x),
20 U RiboLock™ Ribonuclease Inhibitor
20 U T7 RNA polymerase (one unit of the enzyme incorporates 1 nmol of AMP into a polynucleotide fraction in 60 minutes at 37°C.)
in DNase- and RNase-free H_2O.

The samples were incubated for 2 h at 37 °C. The *in vitro* synthesized antisense RNA was cleaned up using the RNeasy Mini Kit (Qiagen) according to the instructions of the manufacturer. For the final elution step, 150 µL RNase-free H_2O was used. A second elution step with 50 µL of the eluate was performed to increase the concentration of the sample. The sequences of the generated [α-^{32}P]-labeled antisense RNA probes are given in the Appendix (Tab. 10.5).
Radioactivity of [α-^{32}P]-labeled antisense RNA probes were determined by liquid scintillation counting of 1 µL RNA probe samples. The samples were combined with 10 mL Rotiszint scintillation cocktail (Roth GmbH; Karlsruhe, Germany) and counted using a Wallac Liquid Scintillation Counter Model 1409 (PerkinElmer; Rodgau, Germany). The probes were directly applied for hybridization (2 x 10^5-2 x 10^6 cpm mL^{-1} hybridization solution).

2.6.4.5. *Hybridization of blotted RNA with [α-^{32}P]-CTP labeled probes*

Pre-hybridization and hybridization of the RNA blots were carried out in UltraHyb solution (Applied Biosystems/Ambion; Darmstadt, Germany). The solution was pre-heated and the blots were pre-hybridized in 15 mL 100 cm^{-2} for at least 1 h at 68-70 °C (blots for hybridization with probes against 16S rRNA were pre-hybridized at 80 °C) in a hybridization oven on a rocking platform (horizontal agitation). For the following hybridization, the probes were denatured by incubation at 100 °C for 10 min, quickly chilled on ice and applied to 15 mL of fresh pre-heated UltraHyb solution. The RNA blots were hybridized with the radiolabelled RNA probes overnight. Unbound probes were removed by stringency washes. First washes were performed two times for 5 min at RT using low-stringency buffer (2 x SSC, 0.1% SDS) to remove the hybridization solution and not hybridized probe. The following high stringency washes (0.5-0.1x SSC, 0.1% SDS) were performed at 68-70°C two times for 15 min to remove partially unspecific hybridized molecules.

2.6.4.6. *Detection and quantification of RNA-RNA hybrids*

The detection of the RNA-RNA hybrids was carried out by phosphorimaging autoradiography. Therefore, the blots were wrapped in cellophane and exposed to a BAS cassette 2025 containing an imaging plate (Fujifilm; Tokyo, Japan) at -80 °C for empirically determined exposure times. Imaging and analyses were performed using a phosphorimager (Image Reader FLA 5000, V2.1; Fujifilm; Tokyo, Japan). Data

processing and quantization was performed using AIDA software (Fujifilm; Tokyo, Japan).

2.6.5. Whole genome microarray analyses

Profiling of relative mRNA abundance under certain growth conditions was performed on a global genome scale by microarray analyses. In general, cDNA synthesized by reverse transcription of total RNA derived from cultures grown under two different conditions, A and B, were separately labeled. The used reverse transcriptase incorporates fluorescence dye labeled deoxynucleotides into the growing cDNA strands. In this work, a so-called two-dye labeling was performed, meaning that cDNA synthesized from total RNA derived from cultures grown under condition A were labeled with another fluorescence dye than the cDNA made from total RNA derived from growth condition B. This allows one to compare the relative amounts of mRNA for several genes isolated from two different conditions simultaneously after hybridization of these labeled cDNA on a microarray that contains immobilized DNA sequences e.g. from a certain organism at known positions (designated as “spots”). To determine the relative mRNA distribution for certain genes between the two growth conditions, the hybridized microarray was scanned with a GenePix 4100A scanner (Axon Instruments; Union City, California, USA). Two lasers mounted into the Chip-Reader emit light beams with a wavelength of 532 nm and 635 nm, respectively. The former wavelength specifically excites the Cy3 dye and the latter one the Cy5 dye. In response to the excitation of the fluorescence dyes light with a specific wavelength for each dye is emitted and captured by a photomultiplier. The software of the GenePix 4100A scanner creates two pictures of the chip, each for one of both dyes. The two pictures were uploaded into the GenePix software and put on top of each other. Then, the software calculates the ratios between the fluorescence intensities of both dyes for every spot on the chip, representing one open reading frame (*orf*), respectively. The fluorescence intensities ratios are a measure of the relative mRNA abundance under the analyzed conditions.

2.6.5.1. *Preparation of fluorescence dye labeled cDNA targets*

Total RNA derived from cultures grown in the presence of 10 µM $Bi(NO_3)_3$ or 100 µM KH_2AsO_4 (condition A) and from cultures grown in the presence of 30 µM KNO_3 or under standard conditions without any modification (condition B) were subjected to

two separately performed cDNA synthesis reactions. The cDNA syntheses were performed by means of the Amersham CyScribe First-Strand cDNA Labeling Kit (Amersham Bioscience; Freiburg, Germany) according to the manufacturer's instructions. 30 µg total RNA was used as template in each reverse transcription reaction. An internal standard was added to each labeling reaction as well (Spike Mix from the Lucidea Universal Score Card (Amersham Bioscience; Freiburg, Germany)), allowing normalization that considers the differential incorporation of the Cy3 and Cy5 labeled dCTPs into the cDNA, respectively. The labeled cDNA was subsequently purified with the CyScribe GFX Purification Kit (Amersham Bioscience; Freiburg, Germany) according to the manufacturer's manual. For analyses of the transcriptional response to 10 µM $Bi(NO_3)_3$ and 100 µM KH_2AsO_4, five individual microarray experiments were performed for the former condition and one for the latter conditions to get a first insight. One dye swap reaction was performed for both conditions. Here, the cDNA derived from RNA isolated from cultures grown under condition A was not labeled with Cy3 as in the other experiments but with Cy5 and for condition B *vice verse*, ensuring that no false results were obtained caused by different incorporation efficiency of the two fluorescence dyes.

2.6.5.2. *Determination of fluorescence dye labeled dCTPs incorporated into cDNA*

For one microarray hybridization, one cDNA synthesis reaction with Cy3 labeled dCTPs (condition A) and two cDNA syntheses reactions with Cy5 labeled dCTPs (condition B) were pooled due to the empirical discovery that only about 50 % of the Cy5 labeled dCTPs are incorporated relative to the Cy3 labeled nucleotides (Veit et al. 2006). The incorporation of the blended fluorescence dye labeled dCTPs was then determined by UV visible spectrophotometry at λ = 550 nm for Cy3 and λ = 650 nm for Cy5 as described in the instructions of the Amersham CyScribe First-Strand cDNA Labeling Kit (Amersham Bioscience, Freiburg) given below:

"The amounts of Cy3 and Cy5 incorporated into cDNA can be calculated from their respective extinction coefficients, (which are 150 000 mol^{-1} cm^{-1} at λ = 550 nm for Cy3 and 250 000 mol^{-1} cm^{-1} at λ = 650 nm for Cy5) as follows:

pmoles Cy3 or Cy5 in sample = $(A/E)\cdot(1/W)\cdot Z\cdot df\cdot 10^6$

Where:

A = absorbance Cy3 at 550 nm or Cy5 at 650 nm

E = the extinction coefficient for Cy3 or Cy5
Z = original volume expressed in microliters
W = optical path of cuvette expressed in centimeters
df = dilution factor

Incorporation of CyDye = pmol of CyDye in sample / µg of nucleic acid in sample"

2.6.5.3. *Manufacturing of microarray chips*

The DNA microarray chips used in this work contained PCR products, covering 3269 *orfs* (97%) from the methanoarchaeum *Methanosarcina mazei* Gö1 (Deppenmeier et al. 2002). These PCR products were printed in duplicates on the aminosilane coated Type-7* microarray slides (Amersham Bioscience; Freiburg, Germany). A detailed description is given elsewhere (Hovey et al. 2005).

2.6.5.4. *Hybridization of fluorescence labeled cDNA targets with microarray slides*

Hybridization of fluorescence labeled cDNA targets with the respective microarray slides were performed in a Lucidea SlidePro hybridization chamber (GE Healthcare; Freiburg, Germany). Therefore, 50 µL of target cDNA containing at least 120 pmol of both fluorescence dyes was incubated at 95 °C for 2 min followed by rapid cooling on ice. 50 µL Microarray Hybridization Solution, Version 2.0 (GE Healthcare; Freiburg, Germany) as well as 110 µL formamide was added, vigorously mixed and injected into the Lucidea SlidePro hybridization chamber containing the microarray slides. Hybridization was performed over night. The detailed hybridization and wash steps are described elsewhere (Hovey et al. 2005).

2.6.5.5. *Prescan and normalization of hybridized microarrays*

The hybridized microarrays were initially scanned at a low resolution to adjust the intensities of the two laser channels (λ = 532 nm and λ = 635 nm). The gain power of the two laser sources were varied until the ratio the fluorescence intensities of both fluorescence dyes of the first spot of the score card (Lucidia Universal Score Card, GE Healthcare; Freiburg, Germany) were approximately 1:1. The dynamic range was also adjusted by aids of the Lucidia Universal Score Card (GE Healthcare; Freiburg, Germany). Further details can be found in the manufacturer's manual. Finally, the microarrays were scanned at a high resolution (5 µm) twice, once for each laser channel (λ = 532 nm and λ = 635 nm). Computer based normalization and data

mining was performed with GenePix-Pro 6.0 software (Axon Instruments; Union City, California, USA). Under the assumption that only a small percentage of the genes are differentially expressed in the presence of Bi and As, respectively, the fluorescence intensity values derived from one channel was multiplied by a certain factor after background correction so that the arithmetic mean calculated from all fluorescence intensities ratios is equal to one. Statistical values for every spot and its surrounding background were generated and exported into Microsoft Excel® for further analyses.

2.6.5.6. *Processing of microarray raw data*

Data derived from the microarray experiments were erased from the data set if they failed one ore more of the following criteria:

The difference between the median fluorescence intensities of the spot signal and the background signal for Cy3 and Cy5, respectively, has to be greater than zero.

The median fluorescence intensities for Cy3 and Cy5 after background subtraction must exceed one standard deviation of the mean value calculated for the background fluorescence intensities.

The three statistical values, ratio of medians, ratio of means and the regression ratio calculated for the fluorescence intensity distribution of both wavelengths, λ = 532 nm and λ = 635 nm, respectively, must not differ more than 30 percent.

The intensity ratios between the mean λ = 532 nm and λ = 635 nm signal values of the remaining spots were transformed into binary logarithm values (log2). The log2 transformed data were used to calculate mean values and standard deviations of the mean intensity ratios of λ = 532 nm to 636 nm for all *orfs* represented from at least three individual groups including one dye-swap experiment (one group consists of total RNA derived from one culture exposed to metal(loid)s and total RNA derived from one control cultures not exposed to metal(loid)s) unless otherwise indicated. In case of the dye-swap experiment, the mean intensity ratios between λ = 635 nm to

532 nm were used for the calculations. The complete data set is given in the appendix (Tab. 10.3 and Tab. 10.4).

2.7. Biochemical methods

2.7.1. Heterologous expression of MtaA from *M. mazei* in *E. coli*

For detailed studies of methylcobalamin:coenzyme M methyltransferase MtaA, the genes *mtaA1* and *mtaA2* (MM_0176, MM_1070) were cloned into either the pET-15b or the pET-24a vector and were subsequently heterologously expressed in *E. coli* BL21(DE3) using T7 RNA polymerase gene and the T7*lac* promoter of the *E. coli* strain. The genes *mtaA1* and *mtaA2*, cloned into the multiple cloning site of either pET-15b or pET-24a, are under control of the T7*lac* promoter on the plasmid vectors. Hence, the expression is repressed until addition of IPTG.
An *E. coli* BL21(DE3) culture (1 L) carrying the appropriate constructed vector was aerobically propagated at 37 °C under shaking until the culture had reached an optical density (OD_{600}) of 0.5-0.6. Expression was induced by addition 100-200 µM IPTG. Incubation was prolonged for additional 4 h and cultures finally harvested by centrifugation (6000 x g, 30 min, 4 °C). The supernatant was discarded and the pellet stored at -80 °C.

2.7.2. Purification of recombinant *M. mazei* enzymes

Heterologously expressed *M. mazei* enzymes MtaA1 and MtaA2 were isolated from cell pellets of recombinant *E. coli* BL21(DE3). Therefore, the cell pellets derived from *E. coli* expression culture was resuspended in either 4 ml 50 mM HEPES pH 7.0 or in 4 ml 50 mM HEPES pH7, 300 mM NaCl, 10 mM Imidazol, depending on the subsequent purification step (Q-sepharose cation exchange chromatography or Ni-NTA chromatography, respectively). The resuspended cells were disrupted by passing through a French pressure cell at 20000 psi until all cells were completely broken as suggested from microscopic analysis. Cell debris and insoluble fractions were removed by centrifugation (from 14000 x g up to 30000 x g, 30 min, 4 °C). The supernatant was collected and dialyzed in a 200-500 fold excess of the respective buffer (4 mL 50 mM HEPES pH 7.0 or 4 mL 50 mM HEPES pH 7, 300 mM NaCl, 10 mM Imidazol) at 8 °C overnight. The dialyzed crude extract was than purified either

on a 10 mL Q-Sepharose FastFlow (GE Healthcare; Freiburg, Germany) containing chromatography column (anion exchange) or on a 4 mL Ni-NTA agarose (Qiagen; Hilden, Germany) containing chromatography column (affinity chromatography) by fast protein liquid chromatography (FPLC). Fractions were eluded from the anion exchange column by a linear NaCl gradient (from 0 to 1 M NaCl in 100 min) at a flow rate of 1 mL min^{-1}. Enzymes that were overexpressed with an N-terminal His_6-tag were purified via affinity chromatography on a Ni-NTA agarose column and eluded by a linear imidazol gradient (from 10 to 500 mM in 40 min) at a flow rate of 1 mL min^{-1}. Fractions were analyzed by denaturizing SDS-PAGE. Fractions containing enzymes of the appropriate size according to SDS-PAGE were pooled and dialyzed in 50 mM HEPES pH7, 300 mM NaCl (with an approx. 100 fold excess of pooled fraction volume). Either the fractions were tested for their catalytic activity or they were further purified by size exclusion FPLC with a Superdex 200 HiLoad 26/60 column (GE Healthcare; Freiburg, Germany), thereby separating enzymes according to their size. In this case, 2-2.5 mL fractions were collected from an isocratic elution at flow rates of 1 mL min^{-1}. An aqueous solution with pH 7 containing 50 mM HEPES and 300 mM NaCl was used for the mobile phase. For size estimation of eluded fragments, a calibration run with enzymes of known sizes was performed. The following size standards were used: Ferritin a (443 kDa), alcohol dehydrogenase (148 kDa), lactate dehydrogenase (78 kDa) and cytochrome c (12.5 kDa). The calibration run was performed by Dipl. Ing. Dipl. Oek. Thomas Knura.

2.7.3. Protein quantification

The determination of protein concentration was carried out using BioRad Protein Assay based on the Bradford protein quantization method (Bradford 1976), following the instructions of the supplier. Bovine serum albumin (BSA; 2-10 µg mL^{-1}) served as standard.

2.7.4. SDS-Polyacrylamide gel electrophoresis (SDS-PAGE)

2.7.4.1. *SDS-PAGE assembling and electrophoresis*

Denaturing sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis (PAGE) was performed to analyze proteins derived from heterologous expression (Laemmli 1970). Therefore, polyacrylamide gels (8.6 cm x 7.7 cm, 1 mm thick) were

used, composed of an upper stacking and a lower separating gel. The acrylamide concentration of the stacking gel (125 mM Tris/HCl, pH 6.8, 0.1% (v/v) SDS, 0.03% (v/v) APS, 0.005% (v/v) TEMED) was 4.9% (v/v) and the concentration of the separation gel (375 mM Tris/HCl, pH 8.8, 0.1% (v/v) SDS, 0.03% (v/v) APS, 0.005% (v/v) TEMED) was 10% (v/v) polyacrylamide. Ingredients for the separating gel were mixed and poured in the gel casting chamber. The gel was covered with H_2O and polymerized for 30 min. The H_2O was then removed and the stacking gel solution was poured upon. A 10-sample well comb was placed into the stacking gel and removed after approximately 45 min. The gels were either directly used for electrophoresis or stored at 4 °C. Prior to electrophoresis, the protein samples were mixed with 5x loading buffer (final concentration: 62.5 mM Tris-HCl, pH 6.8, 10% (v/v) glycerol, 2% (v/v) SDS, 5 % (v/v) β-mercaptoethanol, 0.005% (w/v) bromophenol blue) and heated at 95 °C for 1-2 minutes. Gel runs were performed in a Minigel-Twin-Chamber (Biometra; Göttingen, Germany) containing electrophoresis buffer (25 mM Tris-HCl pH 8, 190 mM glycine and 0.1% (v/v) SDS) at 13 mA for 30 min until the dye front reached the separating gel. The run in the separating gel was continued at 17 mA for 90 min.

2.7.4.2. *Coomassie staining*

Proteins were visualized by gel staining (40% (v/v) methanol, 10% (v/v) acetic acid and 0.25% (w/v) Coomassie Brilliant Blue R-250) for 30 min at 50 °C, followed by destaining (5% (v/v) methanol and 7.5% (v/v) acetic acid) (Weber and Osborn 1969). The gels were documented using the ChemiDoc System in combination with Quantity One Software Package (BioRad Laboratories GmbH; München, Germany).

2.7.4.3. *Molecular mass determination of proteins under denaturing conditions*

The approximate molecular mass of protein subunits was determined by the migration distance in a SDS-PAGE relative to a standard protein mixture (PageRuler^{TM}Unstained Protein Ladder; Fermentas; St. Leon-Rot, Germany). This marker is a mixture of 14 recombinant, highly purified, unstained proteins ranging in size from 10 kDa to 200 kDa. The 50 kDa protein has a clearly greater intensity and served as a reference band.

2.7.5. Determination of methyltransferase activity of recombinant *M. mazei* enzymes

HS-CoM methylation activity of recombinant methyltransferases from *M. mazei* has been determined by two different assays. One assay determines directly the loss of free thiol group of HS-CoM. The other assay was used to determine the rate of CH_3Cob(III) demethylation. Both assays were performed in an anaerobic glove box (H_2/N_2 (2%/98%)) under dim red light to avoid photolytic cleavage of the CH_3Cob(III) C-Co bond. Reactions were started by addition of the recombinant enzyme (10 µg mL^{-1} end concentration) and transfer of the reaction vessel from ice into a thermoblock tempered at 30 °C.

2.7.5.1. *Determination of HS-CoM methylation*

The first assay based on the disappearance of the free thiol moiety of HS-CoM upon its methylation. Aliquots of standard reactions, containing CH_3Cob(III), HS-CoM and the enzyme (buffered at pH 7.0 by 50 mM HEPES), were withdrawn in appropriate time intervals and mixed with a 150 mM Tris/HCl (pH 8) solution, containing 0.5 mM 5,5'-Dithiobis(2-nitrobenzoic Acid) (Ellman's reagent) which reacts specifically with thiol groups, thereby releasing a 2-nitro-5-thiobenzoate anion. This anion has a specific absorption maximum at 412 nm. The decrease of the absorption at 412 nm in aliquots withdrawn in the course of the HS-CoM methylation reaction was used as a measure of the decrease of free thiol groups caused by the transfer a methyl group onto the thiol group of HS-CoM. To calculate molar amounts from the absorption at λ = 412 nm a molar absorption coefficient of 14.15 μM^{-1} cm^{-1} was used (Eyer et al. 2003).

2.7.5.2. *Determination of CH_3Cob(III) demethylation*

In the second assay, the loss of the methyl moiety from CH_3Cob(III) was followed photometrically. Aliquots taken at certain time points were mixed with 10 mM KCN (buffered at pH 10 in a 50 mM potassium borate solution). The samples were oxidized by air to turn reduced cob(I)alamin, formed upon methyl transfer from CH_3Cob(III) to HS-CoM, into oxidized HOCob(III). HOCob(III) rapidly reacts with KCN to form dicyanocobalamin, whereas remaining CH_3Cob(III) is unaffected. The methylation activity was calculated on the basis of the specific absorbance of

dicyanocobalamin at λ = 367 nm with a molar extinction coefficient of 20.87 mM^{-1} cm^{-1} as given by David Grahame (Grahame 1989).

2.7.6. *In vitro* formation of methyl and hydride metal(loid) derivatives

The capability of cell-free crude extracts prepared from *M. mazei* cultures and of recombinant MtaA from *M. mazei* to form methyl and hydride metal(loid) derivatives were analyzed by *in vitro* assays. The reaction mixtures of these *in vitro* assays were prepared in brown 10 mL vials wrapped in aluminum folia. Preparation was carried out in an anaerobic glove box (H_2/N_2 (2%/98%)) to ensure anoxic conditions. The reaction mixtures (1 mL) consists of 1 mM $CH_3Cob(III)$, 1 mM HS-CoM, the respective metal(loid) salt (Tab. 2.8) in concentrations as indicated in the result section and either cell-free crude extract (100 µg mL^{-1}) or purified recombinant MtaA (10 µg mL^{-1}), unless otherwise indicated. The assays were buffered at pH 7 by 50 mM HEPES and air tight sealed by butyl rubber stopper prior to performance of the assays outside the anaerobic glove box. Exposure to light was avoided during the whole preparation as well as during the performance of the assays to prevent photolytic cleavage of the carbon-cobalt bond between the methyl moiety and the cobalt atom of $CH_3Cob(III)$.

Tab. 2.8: List of used metal(loid) compounds.
Synth: Synthesized in the Institute of Environmental Analysis, University of Duisburg-Essen, Essen, Germany.

Compound	Oxidation No. of the metal(loid)	purchased from
$AsNaO_2$	III	Fluka (Buchs; St. Gallen, Schweiz)
CH_3AsI_2	III	Synth.
KH_2AsO_4	V	Sigma Aldrich (Deisendorf, Germany)
$CH_3AsO(ONa)_2$	V	Argus Chemicals (Vernio, Italy)
$(CH_3)_2AsO(OH)$	V	Strem Chemicals, Inc. (Newburyport, MA, USA)
H_2SeO_3	IV	Sigma Aldrich (Deisendorf, Germany)
H_2SeO_4	VI	Sigma Aldrich (Deisendorf, Germany)
$SbCl_3$	III	Sigma Aldrich (Deisendorf, Germany)
$K[Sb(OH)_6]$	V	Sigma Aldrich (Deisendorf, Germany)
Na_2TeO_3	IV	Sigma Aldrich (Deisendorf, Germany)
H_6TeO_6	VI	Sigma Aldrich (Deisendorf, Germany)
$C_3H_5O(COO)_3Bi$	III	Sigma Aldrich (Deisendorf, Germany)
$Bi(NO_3)_3$	III	Sigma Aldrich (Deisendorf, Germany)

2.7.7. UV-Vis spectral analyses

The demethylation of CH_3Cob(III) and its transformation into reduced cob(I)alamin or cob(II)alamin during the reactions catalyzed by MtaA was followed by UV-Vis. Reaction mixtures were prepared in an anaerobic glove box as described in section 2.7.6 but with CH_3Cob(III) concentration <100 µM. The solutions were prepared in quartz cuvettes which were air-tight sealed with a silicon septum harboring screw-cap prior to UV-Vis measurements outside the anaerobic glove box. Reactions were started by placing the cuvettes into a heated cuvette holder (30 °C) of the spectrometer and adding the enzyme. Spectra from λ = 200-700 nm were measured.

2.8. Instrumental analytical techniques

2.8.1. Analysis of volatile metal(loid) derivatives

The formation of volatile metal(loid) species was investigated by purge&trap gas chromatography (PT-GC) connected to an inductively coupled plasma mass spectrometer (ICP-MS) for element specific detection according to Wickenheiser et al. (Wickenheiser et al. 1998). Two different ICP-MS were used in this work. The majority of analyses were performed with an ELAN 6000 (Perkin Elmer; Rodgau, Germany). For analyzes of arsenicals in solution by hydride generation (HG) PT-GC-ICP-MS (described in section 2.8.3.1) a 7500a ICP-MS (Agilent; Yokohama, Japan) was used. The used ICP-MS operating settings are depicted in Tab. 2.9.

Tab. 2.9: ICP-MS operating settings and parameters.

Instruments Parameter	
ICP-MS	7500a (Agilent; Yokohama, Japan)
Nebulizer	MicroFlow PFA nebuliser 100 mL
	(Agilent; Yokohama, Japan)
Spray chamber	Scott-type double pass
Nebuliser carrier gas flow	0.7 L min^{-1}
Nebuliser makeup gas flow	0.32 L min^{-1}
peristaltic pump velocity	0.08 rps
RF power	1520 V
ICP-MS	ELAN 6000 (Perkin Elmer; Rodgau, Germany)
Nebulizer	Ryton nebulizer GemTip™ Cross-Flow II
	(PerkinElmer; Rodgau, Germany)
Spray chamber	Scott spray chamber
Nebuliser carrier gas flow	0.85 L min^{-1}
peristaltic pump velocity	5.8 rps
RF power	1225 V

The PT-GC-ICP-MS measurement routine in brief: Volatile analytes were purged for 10 min by a constant helium carrier gas flow (213 mL min^{-1}) from the reaction vessel via a heated transfer line to a liquid nitrogen cooled chromatographic column (i.d., 4 mm; length, 40 cm; stationary phase, 1.15 g of 10% SP-2100 on 80/100 mesh Supelcoport (Sigma-Aldrich; St. Louis, Missouri, USA)) for cryo-focussing. Then, the column was heated and the analytes eluded under a constant helium flow

(86.25 mL min^{-1}) according to their specific boiling point. The analytes were transferred via a second heated transfer line to the ICP-MS. Here, the analytes were ionized, ions focused by an electric lens system and separated in a quadrupole according to their mass to charge ratio prior to detection by an electron multiplier. The derived data were than analyzed with Microsoft Excel® and Origin 5.0 (OriginLab Corporation; Northampton, MA, USA).

To consider variation in ICP-MS sensitivity over the period of measurement, three internal standards (^{71}Ga and ^{205}Tl, 10 µg L^{-1} each and ^{113}In, 100 µg L^{-1}, solved in 1% (v/v) HNO_3) were introduced into the GC-flow via a Ryton nebulizer (GemTip™ Cross-Flow II; PerkinElmer; Rodgau, Germany) via a Scott spray chamber (PerkinElmer; Rodgau, Germany). The internal standards were amended by 2% propanol or 5% propanol for measurements using the ELAN 6000 or the 7500a ICP-MS, respectively, to minimize fluctuations of the argon plasma caused by introduction of carbon via the GC-stream. In order to calculate the amount of volatile metal(loid) derivatives from the integrated peak area derived for the accordant metal(loid) species, a multielement standard was introduced into the ICP-MS over a nebulizer in a separate run (Tab. 2.10).

Tab. 2.10: Composition of multielement standard solution for determination of the relative standard factor (RSF).
The elements were applied in aqueous solution containing 1% (v/v) of a 65% HNO_3 solution.

Element	Concentration [ppb]	Element	Concentration [ppb]
Ga	10	As	100
Y	10	Se	100
Li	10	Ge	10
Rh	10	Sn	10
Cs	10	Sb	10
Ce	10	Te	10
Tl	10	Hg	10
In	100	Pb	10
		Bi	10

From the derived data the relative standard factor (RSF) was calculated and used together with the nebulizer flow rate and the nebulizer efficiency (calculated from differential weighing of the aspired internal standard solution and the spray chamber drain) to determine total amounts of analyzed metal(loid) derivatives from the ICP-MS signals. The calculations were performed in accordance to the formula for

determination of the RSF and for calculation of the total amount the analyte m_x given by Feldmann (Feldmann 1997):

$$RSF_x = \frac{(I_x - I_{xo}) \cdot c_s}{(I_s - I_{so}) \cdot c_x}$$

with:

RSF_x = relative standard factor of the analyt x

I_x = signal intensity of the analyt x in the multielement standard solution

I_{x0} = signal intensity of the analyt x in the blank (1% (v/v) HNO_3)

I_s = signal intensity of the standard s in the multielement standard solution

I_{s0} = signal intensity of the standard s in the blank (1% (v/v) HNO_3)

x = As, Se, Sb, Te or Bi

s = Ga, Tl or In

$$m_x = \int_{t1}^{t2} \frac{I_x}{I_s} \cdot \frac{v \cdot z}{RSF_x} \cdot dt$$

with:

m_x = amount of analyt

t = time [s]

t1, t2 = peak width

I_x = signal intensity of the analyt x

I_s = signal intensity of the standard s

v = flow rate of the standard s containing solution [mL s^{-1}]

z = nebulizer efficiency (about 6- 8% in this study)

RSF_x = relative standard factor of the analyt x

x = As, Se, Sb, Te or Bi

s = Ga, Tl or In

The standard error of this calibration method was ±30% (Feldmann 1997).

2.8.2. Determination of gas chromatographic retention times of volatile standards

In order to identify the volatile metal(loid) derivatives analyzed by PT-GC-ICP-MS in this thesis, retention times of standards (Tab. 2.11) were determined and plotted

against the boiling points of these compounds as derived from the literature (Fig. 2.2). Volatile metal(loid) species formed in the experiments performed in this work were identified by comparing their retention times to the retention times of the respective metal(loid) derivative standard.

Tab. 2.11: Boiling points of standard compounds.

Compound	retention time measured [s]	Boiling Point [°C]	Reference
AsH_3	44.6 ± 1.0	-45	(Feldmann and Hirner 1995)
SbH_3	64.7 ± 3.6	-16.0	(Feldmann and Hirner 1995)
CH_3AsH_2	89.1 ± 4.8	3.0	(Feldmann and Hirner 1995)
pentane (C5)	127.7 ± 3.6	36.1	(Jesson et al. 2006)
$(CH_3)_3As$	140.9 ± 8.2	52.0	(Dyke and Jones 1930)
$(CH_3)_2Se$	156.3 ± 4.1	57.7	(Graham and Stone 1956)
hexane (C6)	176.6 ± 3.6	68.7	(Jesson et al. 2006)
heptane (C7)	239.0 ± 5.1	98.4	(Jesson et al. 2006)
$(CH_3)_2S_2$	253.1 ± 2.7	110.0	(Swearingen et al. 2004)
octane (C8)	314.7 ± 2.1	125.7	(Jesson et al. 2006)
$(CH_3)_2Se_2$	344.6 ±1.3	153.0	(Paetzold et al. 1960)
nonane (C9)	372.5 ± 2.1	150.8	(Jesson et al. 2006)
decane (C10)	424.7 ± 5.9	174.1	(Jesson et al. 2006)

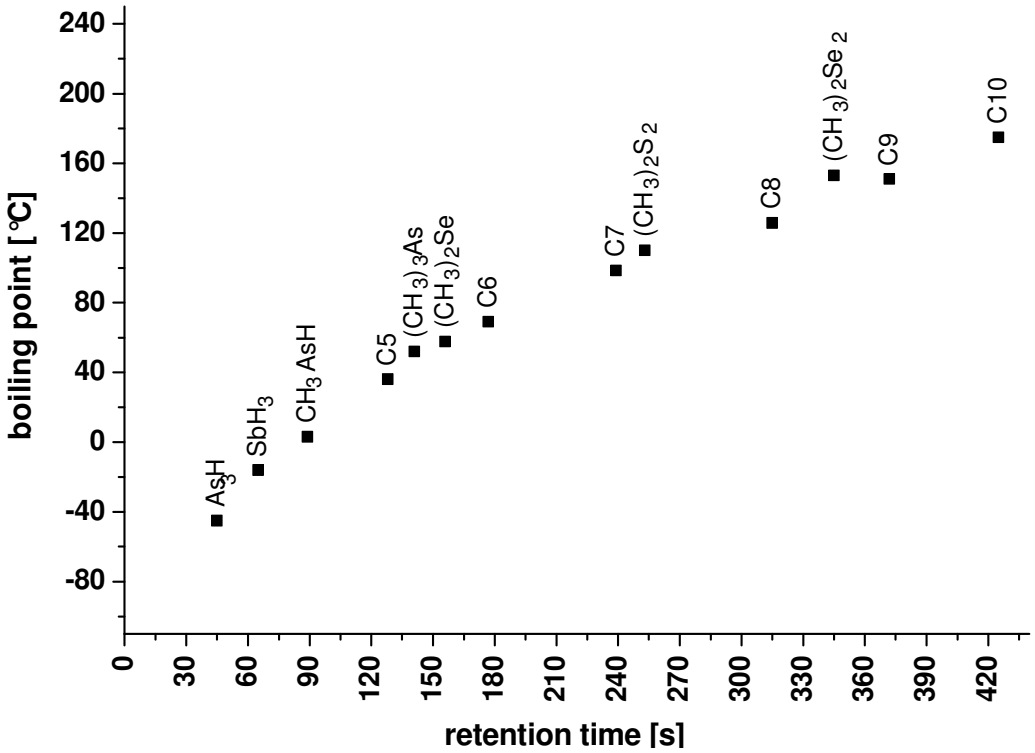

Fig. 2.2: Boiling point retention times of standard compounds subjected to PT-GC-ICP-MS. Volatile standards were either directly introduced into the PT-GC apparatus via an injection port or previously formed from non-volatile standards by hydride generation using sodium tetrahydroborate. C5 to C10 designates alkanes from pentane to decane. The according boiling points are derived from literature Tab. 2.11.

2.8.3. Analyses of non-volatile methylated metal(loid) derivatives

In order to analyze non-volatile methylated As and Bi derivatives, samples were subjected to chemical derivatization reactions. Soluble arsenic derivatives were hydrogenated and soluble bismuth derivatives were ethylated.

2.8.3.1. *Chemical derivatization of volatile As hydrides*

For analyses of methyl arsenicals in solution by hydride generation (HG) PT-GC-ICP-MS, a 7500a ICP-MS (Agilent; Yokohama, Japan) from the Institute of Environmental Analysis (University of Duisburg-Essen; Essen, Germany) was used. Non-volatile methylated As derivatives were chemically hydrogenated by sodium tetrahydroborate. The proceeding was semi-automatized as described by Diaz-Bone and Hitzke 2008 (Diaz-Bone and Hitzke 2008). Optimization of the reaction conditions and software based automation of the proceeding was done by Diploma Chemist Oliver Würfel in the course of his PhD thesis.

In brief, aliquots of the *in vitro* assays (section 2.7.6) were buffered at pH 7-8 in 50 mL Na_3 citrate buffer (pH 7) or 50 mM Tris/HCl buffer (pH 8), respectively,

provided in a 100 mL four-neck round-bottomed flask. Oxygen was removed from the solution by purging the reaction mixture 0.5-2 min with He. Derivatization was started by continuous addition of a 1 M $NaBH_4$ solution, resulting in the hydride generation and thereby volatilization of trivalent soluble As derivatives. Then, the reaction mixture was stepwise acidified by addition of 1 M hydrochloric acid during the prolonged $NaBH_4$ addition, resulting in hydride generation of pentavalent As compounds (pH-gradient). The formed volatile hydride derivatives were removed from the reaction vessel by a continuous He carrier gas flow and transferred via a heated transfer line to a liquid nitrogen cooled chromatography column for cryo-trapping. In order to distinguish between tri- and pentavalent As derivatives the hydride species formed at pH 7-8 were trapped on one column (trivalent derivatives) and the derivatives formed during the pH gradient on a second column (pentavalent derivatives). The cryo-trapped As species were eluted from the column according to their boiling points during a defined heating program. The analytes were than transferred by a continuous He flow to an ICP-MS for detection.

All arsenic reactants used were initially subjected to chemical hydride generation in the same dilution as in the assays in order to determine their concentration and to check purity with respect to arsenic derivatives of other oxidation state and methylation grade. The results of these analyses are listed in Tab. 2.12. With regard to the compounds expected, recovery of non-expected derivatives (false positive) caused by impurity of the arsenic standards or by spontaneous auto-transformation were <7%. The arsenic reactant standards used were measured at the beginning, at the end and in between of each trial in order to check the stability of the reactants. The arsenical standards (reactants) were stable as concluded from the low relative standard deviations (1-6%) derived from the measurements of the reactant standards at different time points.

Tab. 2.12: Determination of concentration and purity of different arsenic compounds by pH dependent HG-PT-GC-ICP-MS analyses.
Arsenic standards were subjected to chemical hydride generation by sodium borhydride at pH 7-8 and during pH gradient from pH 8 to 1 in order to differentiate between trivalent and pentavalent arsenic derivatives, respectively. The volatilized arsenic derivatives were analyses by PT-GC-ICP-MS. The ratio between the amounts of volatile derivatives (minus the hydrogenated form of the applied arsenic standard) to the hydrogenated arsenic standard is given as rate of false positive. Hydride generation efficiency was >90% (Oliver Würfel, personal communication). Experiments were performed in triplicates.

		$AsNaO_2$	$CH_3AsO(ONa)_2$	CH_3AsI_2	$(CH_3)_2AsO(OH)$
total volatilized [pmol] (± %SD)		9 304 (± 2%)	57 636 (± 5%)	877 (± 2%)	1 616 (± 2%)
rate of false positive [%]		2	1	6	5
amounts of methylated and hydrogenated arsenic derivatives as detected by HG-PT-GC/ICP-MS analyses [pmol] (±%SD)					
AsH_3	pH 7 - 8	**9 158 (± 1%)**	31 (± 99%)	4.4 ([± 11%)	4.0 (± 11%)
	gradient	128 (± 21%)	<DL	35 (± 74%)	54 (± 2%)
CH_3AsH_2	pH 7 - 8	<DL	92 (± 1%)	**798 (± 2%)**	1.8 (± 32%)
	gradient	4.6 (± 21%)	**57 432 (± 6%)**	8.3 (± 78%)	14 (± 15%)
$(CH_3)_2AsH$	pH 7 - 8	<DL	<DL	<DL	7.2 (± 20%)
	gradient	6.1 (± 28%)	<DL	3.5 (± 74%)	**1 528 (± 2%)**
$(CH_3)_3As$	pH 7 - 8	<DL	<DL	<DL	<DL
	gradient	1.5 (± 8%)	<DL	1.0 (± 76%)	5.7 (± 110%)

<DL: below dection limit.

2.8.3.2. *Chemical derivatization of volatile Bi ethyl compounds*

Formation of partly methylated non-volatile Bi derivatives was analyzed by PT-GC-ICP-MS headspace analyses after chemical derivatization of volatile bismuth ethyl derivatives by using sodium tetraethyl borate according to Hollmann and coworker (Hollmann et al. 2010). Samples were diluted in water to yield approx. 1.0-0.1 µM total Bi. 1 mL diluted sample was mixed in 4 mL phosphate buffer (pH 7) provided in 25 mL glass vials. The reaction vessels were capped with a butyl-rubber septum and oxygen was removed through a cannula (outlet) by purging the solution for 3 min with a continuous He gas flow (93 mL min^{-1}) led through a second cannula. The reaction vessels were than connected via the outlet to a heated transfer line leading to a liquid nitrogen cooled packed chromatography column (stationary phase, 1.15 g of 10% SP-2100 on 80/100 mesh Supelcoport (Sigma-Aldrich; St. Louis, Missouri, USA)). 1 mL of a 0.1% sodium tetraethyl borate solution (stabilized in 1% NaOH) was slowly added by a syringe to the reaction mixture in order to form volatile Bi ethyl derivatives, removed by a continuous He flow for 15 min and cryo-trapped on the

liquid nitrogen cooled packed chromatography column. The cryo-trapped Bi species were eluted from the column by heating according to their boiling points. The analytes were than transferred by a continuous He flow to an ICP-MS for detection.

3. Results

3.1. Studies on the formation of methyl and hydride metal(loid) compounds

3.1.1. Formation of volatile methyl and hydride metal(loid) derivatives accelerated by *M mazei* cell crude extract

The finding that almost all tested methanoarchaea are versatile in methylating various metal(loid)s hints to a connection between methanogenesis and metal(loid) methylation (Meyer et al. 2008). In this thesis, it was analyzed whether these volatile methyl metal(loid) derivatives formed by *M. mazei* grown in the presence of elevated metal(loid) concentrations of As, Se, Sb, Te and Bi can also be generated by *M. mazei* without induced expression of specific metal(loid) methylating enzymes. Therefore, metal(loid) methylation capability of cell-free crude extracts prepared from methanol grown *M. mazei* cultures not pre-incubated with elevated metal(loid) concentration was investigated by PT-GC-ICP-MS. The cell-free crude extracts were freshly prepared, not subjected to intensive dialyzation steps and stored at 8 °C for no longer than four days to prevent the degradation of the proteins. The performed *in vitro* assays contained 100 µg mL^{-1} cell-free crude extracts and 1 mM $CH_3Cob(III)$. $CH_3Cob(III)$ is a verifiable methyl group donor for arsenic and bismuth methylation in methanoarchaea (McBride and Wolfe 1971; Michalke et al. 2002). The assays were additionally amended by a key cofactor of methanogenesis, HS-CoM, which is the main methyl group acceptor in $CH_3Cob(III)$ dependent transmethylation reactions in all methanogenic pathways (see section 1.1.4.4). It was tested whether these *in vitro* assays are capable to form methyl and hydride derivatives of arsenic and bismuth like in the studies of McBride and Wolfe as well as Michalke and coworker (McBride and Wolfe 1971; Michalke et al. 2002) and whether additionally the metal(loid)s selenium, antimony and tellurium are converted into volatile methyl and hydride derivatives.

Notable amounts of volatile metal(loid) compounds were detected in the headspace of the reaction vessels within 10 min upon addition of metal(loid)s to the *in vitro* assays (Tab. 3.1).

Tab. 3.1: Multi-metal(loid) methylation and hydride generation capability of soluble crude extracts prepared from *M. mazei* cultures not exposed to elevated metal(loid) concentrations.
Detected amounts of volatile metal(loid) derivatives formed at 30 °C by *in vitro* assays containing 1 µmol $CH_3Cob(III)$ and HS-CoM, the respective metal(loid) (as indicated) and 1 mL 50 mM HEPES pH 7 are shown. Reactions were started by addition of 100 µg soluble *M. mazei* crude extract or by incubation at 30 °C alone (negative control) for 10 min prior to headspace analyses by PT-GC-ICP-MS. Mean values and relative standard deviations (%SD) of the formed volatile metal(loid) derivatives (boldface) and percentile conversion (%) are presented. Experiments were performed in triplicates. Detection limit calculations based on the standard deviation of the respective signal background noise.

element		As(III)	Se(IV)	Sb(III)	Te(IV)	Bi(III)
reactant		$AsNaO_2$	H_2SeO_3	$SbCl_3$	Na_2TeO_3	$C_3H_5O(COO)_3Bi$
total amount added	[pmol]	100	10 000	100	10 000	100
total volatilized	[pmol] (±%SD)	**25 (±46%)**	**1 270 (±65%)**	**35 (±29%)**	**1 030 (±50%)**	**14 (±74%)**
	%	25	13	35	10	14
negative control (without protein)	[pmol] (±%SD)	**<0.0049†**	**14 (±3%)**	**<0.0039†**	**<0.0074†**	**0.0031(±42%)**
	%	<0.0049†	0.14	<0.0039†	<0.0001†	0.0031
detection limit	[pmol]	0.0049	0.12	0.0039	0.0074	0.0002

†below detection limit

The formed volatile metal(loid) species were identified as AsH_3, CH_3AsH_2, $(CH_3)_2AsH$, $(CH_3)_3As$, As_u (unknown arsenical), CH_3SeH, $(CH_3)_2Se$, $(CH_3)_2Se_2$, $(CH_3)_2SbH$, $(CH_3)_3Sb$, $(CH_3)_2Te$, $(CH_3)_2Te_2$ and $(CH_3)_3Bi$ according to their boiling point retention times (Fig. 3.1). In the case of arsenic, the formation of hydride species exceeds the formation of the permethylated $(CH_3)_3As$. Also high quantities of CH_3SeH, formed from selenite, were detected.

Early studies suggest that $CH_3Cob(III)$ dependent arsenic and bismuth methylation is coupled to enzymatically catalyzed reactions (McBride and Wolfe 1971; Michalke et al. 2002). As in this thesis the used $CH_3Cob(III)$ concentration exceeded the concentration used in the early studies performed with cell-free crude extracts prepared from *Mb. bryantii* and *Mb. formicicum* 100 and 1000-times, respectively, it was tested whether under this condition involvement of enzymes is dispensable. When $CH_3Cob(III)$ and HS-CoM were incubated with the Group 15 and 16 metal(loid)s covered in this thesis in the absence of the cell-free crude extracts, a more than four orders of magnitude lower conversion of inorganic metal(loid)s into volatile metal(loid) methyl and hydride derivatives was observed (Tab. 3.1). Formation of mentionable amounts of volatile methyl and hydride derivatives without enzyme were only found in the case of selenium. However, the amounts detected were 90-times lower relative to assays containing cell-free crude extracts. Hence, increasing the $CH_3Cob(III)$ concentration does not enable abiotic metal(loid) methylation and hydride generation. Enzymes constitutively expressed in methanol

grown *M. mazei* cells are obviously required in the presence of CH_3Cob(III) and HS-CoM to form volatile methyl and hydride derivatives of Group 15 and 16 metal(loid)s.

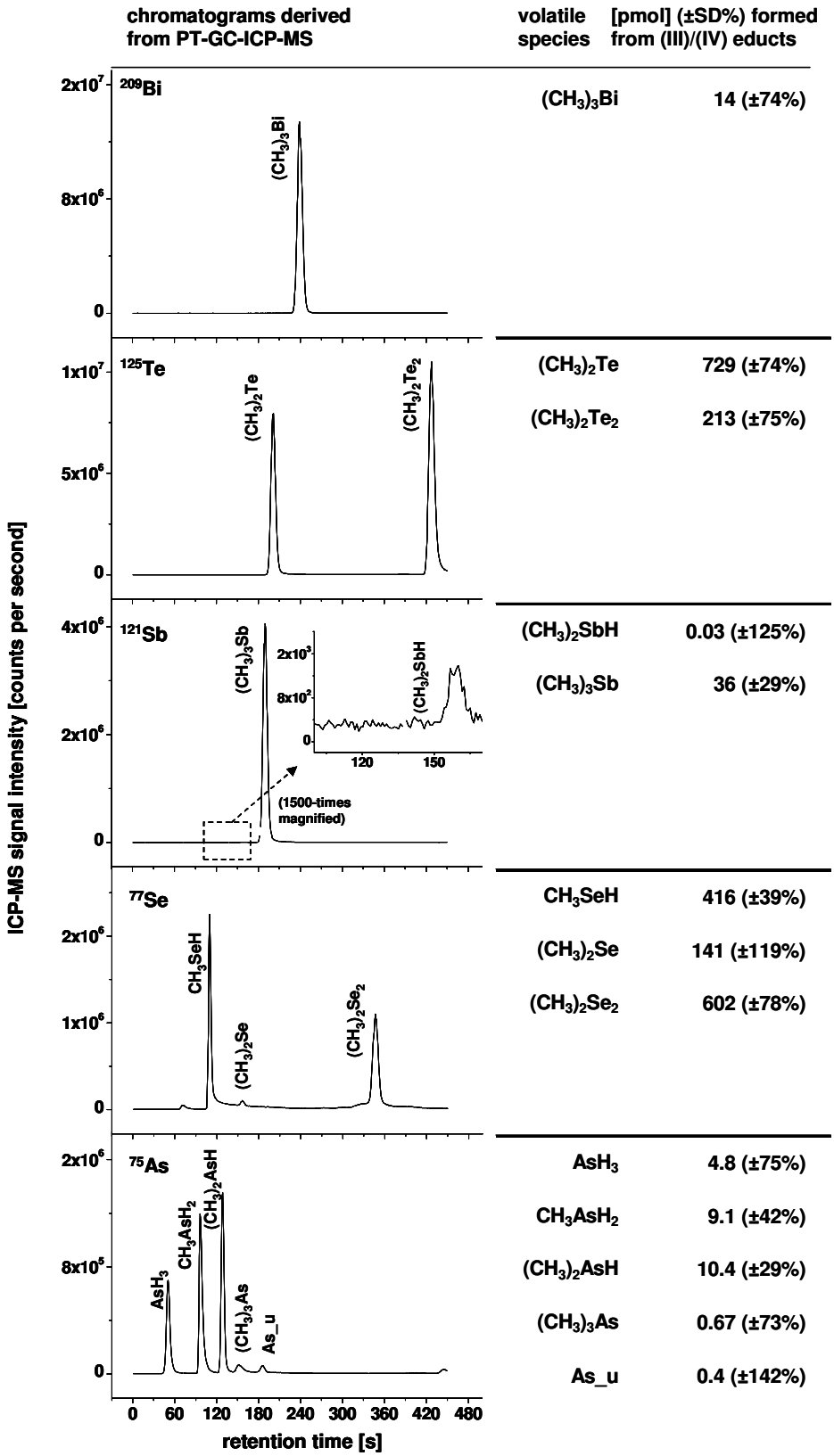

Fig. 3.1: Crude extracts prepared from *M. mazei* triggers metal(loid) methylation and hydride generation in the presence of CH_3Cob(III) and HS-CoM.
Headspace PT-GC-ICP-MS chromatograms of reaction mixtures containing 1 µmol CH_3Cob(III) and HS-CoM, 0.1 nmol Bi(III), Sb(III), As(III) or 10 nmol Te(IV), Se(IV), and 1 mL 50 mM HEPES pH 7 are shown. Reaction was started by addition of 100 µg soluble crude extract followed by incubated at 30 °C for 10 min. Numerical values are derived from three independent experiments. Relative standard deviations (%SD) are in brackets. As_u: Unknown volatile arsenical.

3.1.2. Preparation of purified recombinant methyltransferase MtaA from *M. mazei*

The performed *in vitro* assays with crude extracts prepared from *M. mazei* cultures not exposed to elevated metal(loid) concentrations point towards involvement of soluble CH_3Cob(III) utilizing methyltransferases in multi-metal(loid) methylation and hydride generation (see section 3.1.1). The methyltransferase is apparently constitutively expressed upon growth on methanol. Hence, the methyltransferase MtaA comes into consideration as it is an integral part of methylotrophic methanogenesis from methanol, catalyzing the CH_3Cob(III) dependent methylation of HS-CoM (see section 1.1.4.4). Two *orf*s on the *M. mazei* genome (MM_0176 and MM_1070) encode MtaA. The two *orfs* MM_0176 and MM_1070 show 78% amino-acid sequence identity. Both *orf*s were cloned and heterologously expressed in *E. coli* followed by purification to allow detailed analyses whether MtaA enables multi-metal(loid) methylation in the presence of CH_3Cob(III) and HS-CoM.

3.1.2.1. *Expression of recombinant methyltransferases of* M. mazei *in* E.coli

Both MtaA encoding *orf*s (MM_0176 and MM_1070) from *M. mazei* were successfully ligated into the plasmid pET-15b and pET-24a, respectively, and transformed into competent *E. coli* cells. After transformation, the recombinant enzymes were heterologously expressed in *E. coli* BL21(DE3), using the IPTG inducible T7 expression system of the pET vector and the T7 polymerase of *E. coli* BL21(DE3). Expression of MtaA encoded by either *orf* MM_0176 or MM_1070 was noticeable 3-4 h after ITPG addition as suggested from the appearance of an approx. 37 kDa band in cell-free crude extracts prepared from *E. coli* BL21(DE3) carrying the vector with inserted *M. mazei orf*s MM_0176 or MM_1070 (Fig. 3.2). The size of these bands matches with the theoretical size of the enzyme. Higher amounts of the recombinant methyltransferases were present in the soluble cell-free crude extract than in the insoluble fraction.

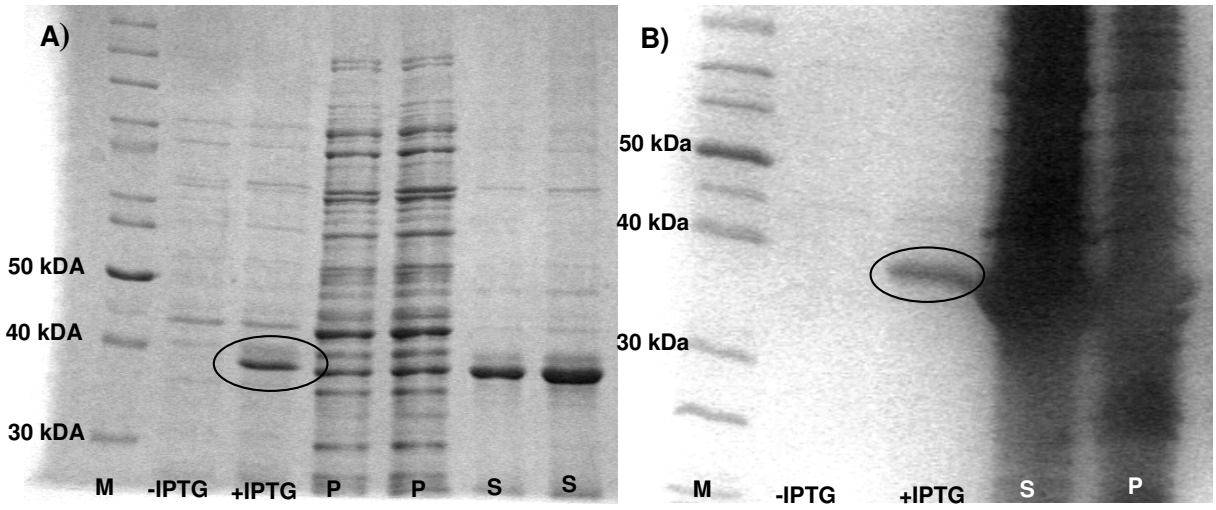

Fig. 3.2: Over-expressed recombinant MtaA from *M. mazei* in *E. coli*.
SDS-PAGEs of recombinant MtaA encoded by *orf* MM_0176 A) and MM_1070 B) from *M. mazei* expressed in *E. coli* BL21(DE3) are shown. M: marker (PageRuler® unstained Protein Ladder, Fermentas), -IPTG: cells before IPTG addition, +IPTG: cells 3 – 4 h after IPTG addition, S: soluble cell crude extract, P: insoluble cell pellet.

3.1.2.2. *Purification of recombinant methyltransferases MtaA*

For preliminary analyses, MtaA (MM_0176 and MM_1070) was heterologously over-expressed with an N-terminal His_6-tag. The soluble fractions derived from the heterolougous expressions of MtaA were purified on a Ni-NTA column (Qiagen; 5 mL), resulting in the elution of an approx. 37 kDa protein fraction at an imidazol concentration between 175 mM and 250 mM as exemplified on MtaA encoded by *orf* MM_0176 (Fig. 3.3). The respective fractions were less than 10% contaminated with proteins of higher or lower molecular mass as suggested from the SDS-PAGE, respectively. Upon combining fractions 14 to 25, an 18 mL protein solution with a concentration of 230 µg mL^{-1} was obtained from the initial 1L *E. coli* expression culture.

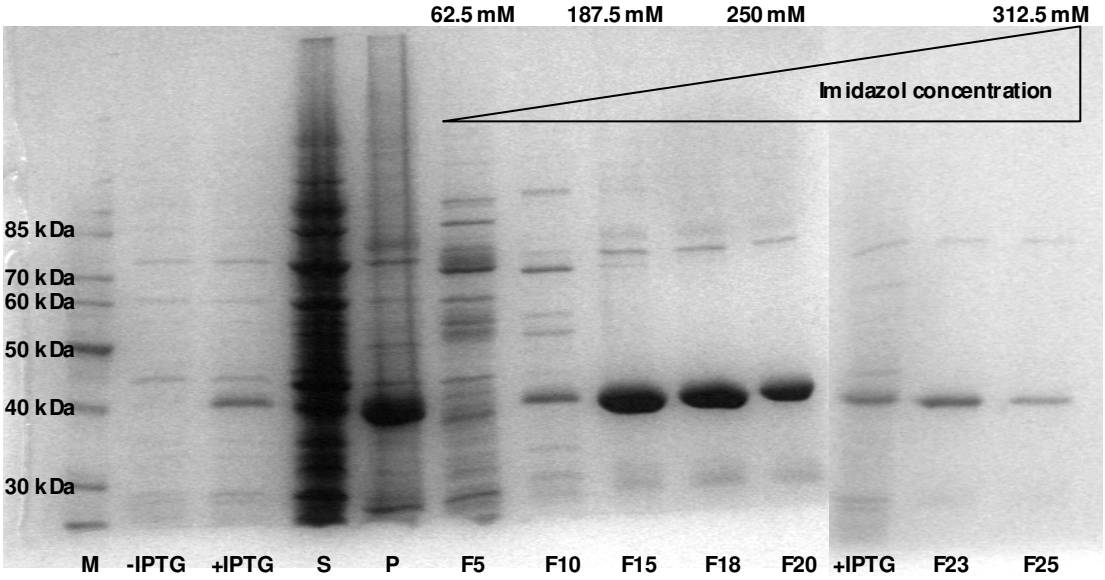

Fig. 3.3: Purification of recombinant MtaA (MM_0176) carrying an N-terminal His_6-Tag by Ni-NTA affinity chromatography.
SDS-PAGE of Ni-NTA affinity chromatography of *E. coli* BL21(DE3) crude extract containing over-expressed His_6-tagged recombinant MtaA (MM_0176) is depicted. The cell crude extract was loaded onto a chromatography column packed with Ni-NTA resin. Fractions were eluted by a linear imidazol gradient (0 to 500 mM in 40 min) at a flow rate of 1 mL min^{-1}. 1 mL fractions were collected. M: marker (PageRuler® unstained Protein Ladder, Fermentas), -IPTG: cells before IPTG addition, +IPTG: cells after IPTG addition, S: soluble cell crude extract, P: insoluble cell pellet, F5: fraction 5, F10: fraction 10 etc..

For the bulk analyses, MtaA encoded by *orf* MM_1070 was heterologously expressed without a His_6-tag to avoid possible influences of the His_6-tag on the metal(loid) methylation and hydride generation. The first purification step by anion exchange chromatography (Q-Sepharose packed column; 10 mL) yielded an approx. 37 kDa protein fraction, eluted at 480 mM sodium chloride (Fig. 3.4). Fractions 23 to 28 were pooled (yielding about 12 mL) and dialyzed in 1 L buffer (50 mM HEPES pH 7.0, 300 mM NaCl, 5 mM DTT) at 8 °C overnight. The dialyzed protein fraction was concentrated using a 10 kDa Vivaspin column to yield 5 mL prior to size exclusion chromatography (HiLoad 26/60 Superdex 200 column). A main protein peak was detected by UV upon eluting about 240 mL running buffer from the size exclusion chromatography column. The fractions corresponding to the detected peak contained a homogenous 37 kDa protein as suggested from SDS-PAGE (Fig. 3.5). The fractions with the homogeneous protein fraction were pooled, diluted to yield 250 µg mL^{-1} and spiked with glycerol (endconc. 3%). 1 mL aliquots were quickly frozen in liquid nitrogen and stored at -20 °C until use. About 15 mg of homogeneous protein was prepared from 1 L *E. coli* expression corresponding to approx. 1.4 g cells wet weight.

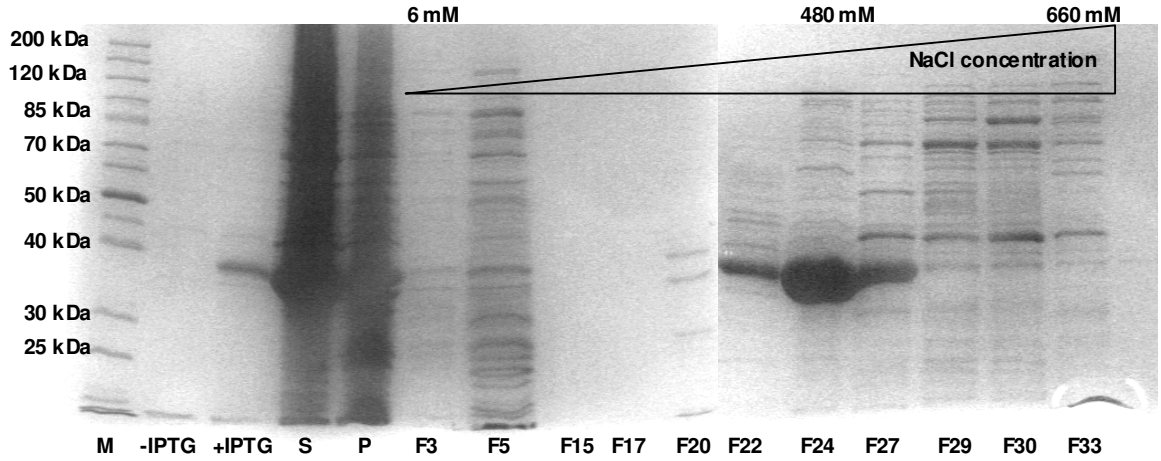

Fig. 3.4: Purification of recombinant MtaA (MM_1070) by Q-Sepharose anion exchange chromatography.
SDS-PAGE of Q-Sepharose anion exchange chromatography of *E. coli* BL21(DE3) cell-free crude extract containing heterologously expressed recombinant MtaA (MM_1070) is depicted. The cell crude extract was loaded onto a chromatography column packed with Q-Sepharose (10 mL). Fractions were eluted by a linear sodium chloride gradient (0 to 1000 mM in 100 min) at a flow rate of 1 mL min^{-1}. 2 mL fractions were collected. M: marker (PageRuler® unstained Protein Ladder, Fermentas), -IPTG: cells before IPTG addition, +IPTG: cells after IPTG addition, S: soluble cell crude extract, P: insoluble cell pellet, F3: fraction 3, F5: fraction 5 etc..

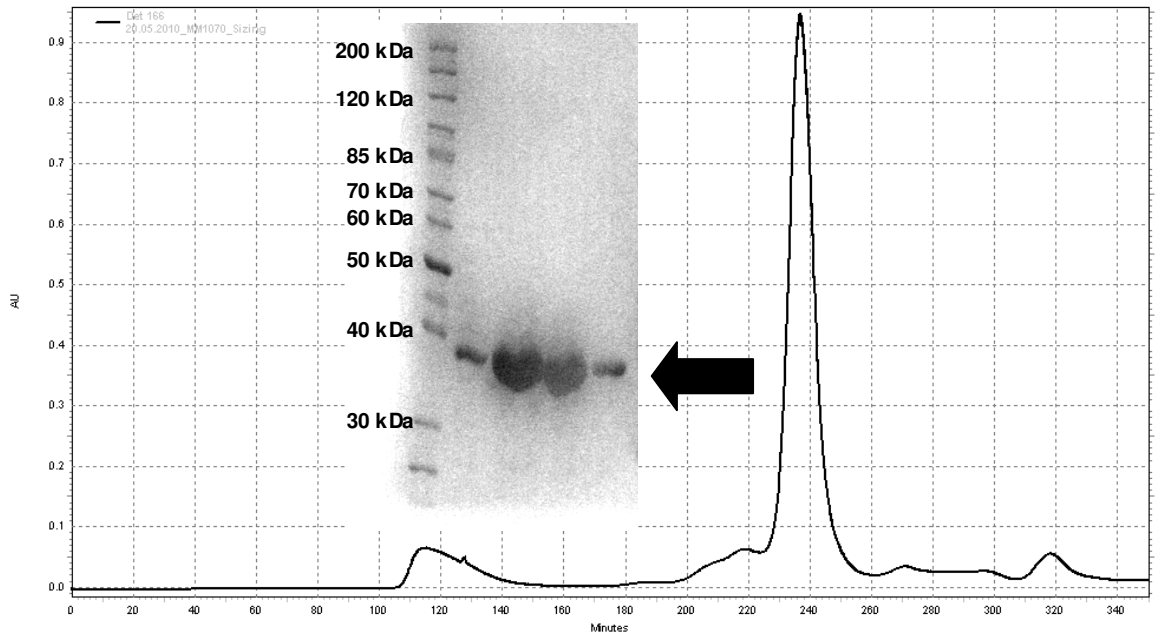

Fig. 3.5: Purification of recombinant MtaA (MM_1070) by size exclusion chromatography.
UV absorption profile and SDS-PAGE of a size exclusion chromatography run of recombinant MtaA (MM_1070) is depicted. 5 mL of a pooled fraction predominantly containing proteins with a molecular mass of 37 kDa derived upon purification of the heterologous expression of MtaA (MM_1070) in *E. coli* BL21(DE3) by Q-sepharose anion exchange chromatography was loaded onto a HiLoad 26/60 Superdex 200 size exclusion chromatography column. 2.5 mL fractions were eluted isocratic at a flow rate of 1 mL min^{-1}. Fractions according to the UV detection peak appearing 240 min after start of the chromatography run were analyzed by SDS-PAGE.

3.1.3. Determination of the HS-CoM methylation activity of MtaA

The recombinant enzymes purified were tested for their $CH_3Cob(III)$ dependent HS-CoM methylation activity to ensure that these enzymes are MtaA. First, the disappearance of the free thiol group of HS-CoM in the course of its methylation was analyzed by using Ellman's reagent (described in section 2.7.5.1). Both recombinant proteins were capable to methylate HS-CoM (Fig. 3.6). A significant disappearance of free thiol groups was detected within five minutes in the presence of 20 µg mL^{-1} recombinant MtaA encoded by *orf* MM_1070 and 1 mM $CH_3Cob(III)$. A decrease of free thiol groups in the reaction mixture was also observed in the presence of MtaA encoded by MM_0176, but less significantly. In contrast, no disappearance of the thiol group of HS-CoM was evident when MtaA was omitted. The data show that the purified recombinant enzymes are indeed MtaA enzymes.

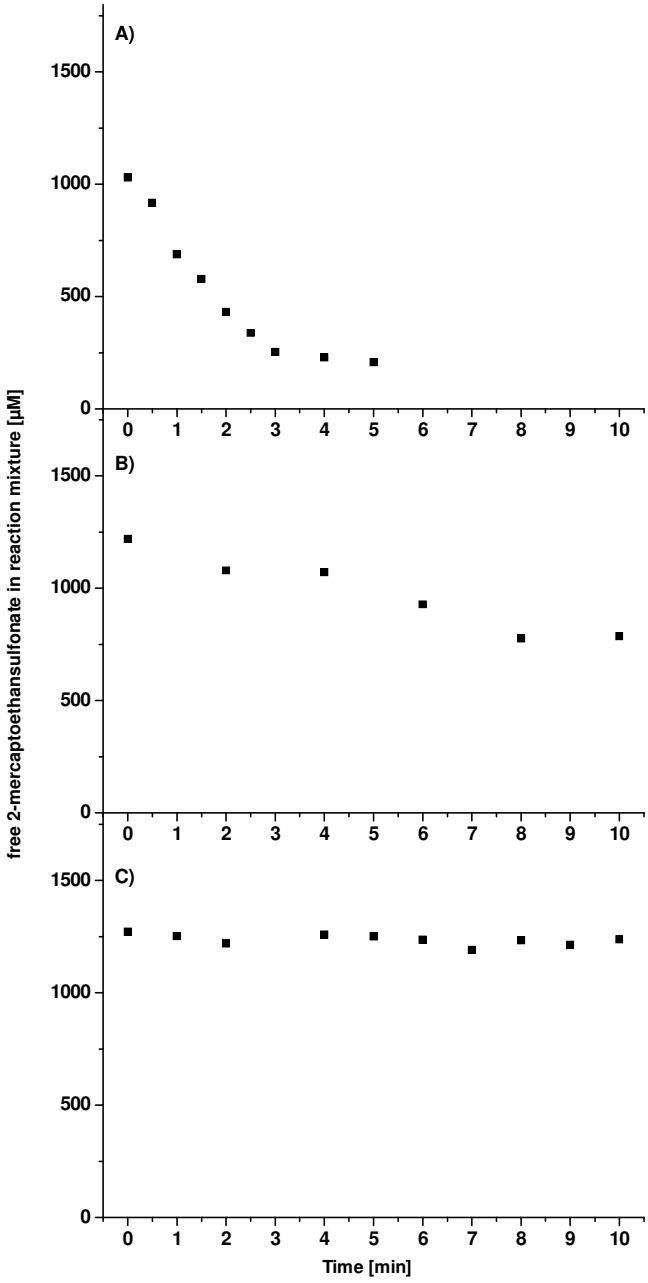

Fig. 3.6: HS-CoM methylation activity of recombinant MtaA from *M. mazei*.
The disappearance of free thiol groups of 2-mercaptoethanesulfonate (HS-CoM) upon its methylation was determined for 20 µg mL^{-1} recombinant MtaA encoded by *orf* MM_1070 A), MM_0176 B) or without protein C) in the presence of 1 mM $CH_3Cob(III)$ at 30 °C. Reaction mixtures additionally contained A) 1 mM HS-CoM or B, C) 1.2 mM HS-CoM.

The reaction mixtures contained high concentrations of the corrinoid $CH_3Cob(III)$ as methyl group donor. The corrinoid changes its specific absorption spectrum upon its demethylation, resulting in the formation of Cob(I) or Cob(II) upon auto-oxidation under less reductive conditions (Kreft and Schink 1994). The corrinoid cofactor Cob(I) and Cob(II) interferes differentially with the specific absorption at 412 nm of

the 2-nitro-5-thiobenzoate anion, released upon reaction of 5,5'-dithiobis(2-nitrobenzoic acid) (Ellman's reagent) with the free thiol groups of non-methylated HS-CoM. Thus, the formed CH_3-S-CoM concentration could not be determined adequately by this technique as the ratio between formed Cob(I) and Cob(II) was unknown. Consequently, the MtaA catalyzed demethylation of CH_3Cob(III) in the course of HS-CoM methylation was followed by cyanide derivatization assays for a more quantitative determination of the transmethylation activity of MtaA as previously described by David Grahame (Grahame 1989).

The demethylation of 1 µmol CH_3Cob(III) in the presence of 1 µmol HS-CoM and 10 µg mL^{-1} MtaA (MM_1070), provided in 1 mL 50 mM HEPES pH 7, at 30 °C is depicted below (Fig. 3.7). 438.4 nmol of CH_3Cob(III) – i.e. 43.8% of the added CH_3Cob(III) - was demethylated under the given conditions within 10 min. Highest specific transmethylation activity (7.3±1.3 U mg^{-1} protein) was detected in the initial first min of the reaction. The methylation rate was centered to a mean value of 4.8±0.7 U mg^{-1} protein over the subsequent five minutes period and decreased to a rate of 3.2±0.1 U mg^{-1} protein in the last four minutes of the measurements.

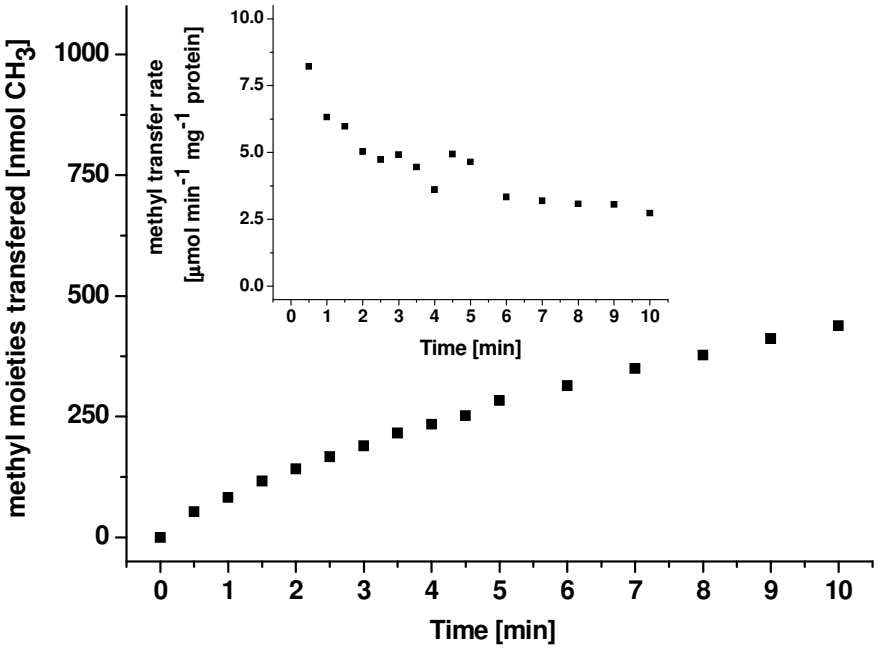

Fig. 3.7: CH_3Cob(III) demethylation activity of recombinant MtaA.
Demethylation of CH_3Cob(III) by 10 µg mL^{-1} recombinant MtaA (MM_1070) is determined in the presence of 1 µmol CH_3Cob(III) and HS-CoM each and 1 mL 50 mM HEPES pH 7 at 30 °C by using cyanide derivatization assays. The methyl transfer rates (in µmol min^{-1} mg^{-1} protein) are given in the inset. Experiments were performed in duplicates.

3.1.4. Analyses of multi-metal(loid) methylation and hydride generation accelerated by MtaA

The *in vitro* assays described in section 3.1.1 were repeated but with 10 µg mL^{-1} purified recombinant MtaA (MM_1070) instead of 100 µg mL^{-1} cell-free crude extract to test whether purified recombinant MtaA can substitute the cell-free crude extract prepared from methanol grown *M. mazei* cultures in the observed multi-metal(loid) methylation and hydride generation reaction. The headspace analyses by PT-GC-ICP-MS of *in vitro* assays containing purified recombinant MtaA, $CH_3Cob(III)$, HS-CoM and different metal(loid)s as listed in Tab. 3.2 demonstrated notable formation of volatile methyl and hydride derivatives of all metal(loid)s applied (Tab. 3.2).

Tab. 3.2: Volatilization of numerous metal(loid)s by purified recombinant MtaA (MM_1070). Detected amounts of volatile methyl and hydride metal(loid) derivatives formed by *in vitro* assays containing 1 µmol $CH_3Cob(III)$ and HS-CoM, the respective metal(loid) and 1 mL 50 mM HEPES pH 7 are given. Reactions were started by addition of 10 µg purified recombinant MtaA (MM_1070) or by incubation at 30 °C alone (negative control without protein) for 10 min, respectively, prior to headspace analyses by PT-GC-ICP-MS. Mean values and relative standard deviations (%SD) of the formed volatile metal(loid) derivatives (boldface) and percentile conversion (%) are presented. Experiments were performed in triplicates. Detection limit calculations based on the standard deviation of the respective signal background noise.

element		As(III)	Se(IV)	Sb(III)	Te(IV)	Bi(III)
reactant		$AsNaO_2$	H_2SeO_3	$SbCl_3$	Na_2TeO_3	$C_3H_5O(COO)_3Bi$
total amount added	[pmol]	100	10 000	100	10 000	100
total volatilized	[pmol] (±%SD)	**43 (±33%)**	**2 989 (±39%)**	**86 (±12%)**	**1 816 (±26%)**	**23 (±2%)**
	%	43	30	86	18	23
negative control (without protein)	[pmol] (±%SD)	**<0.0049†**	**14 (±3%)**	**<0.0039†**	**<0.0074†**	**0.0031(±42%)**
	%	<0.0049†	0.14	<0.0039†	<0.0001†	0.0031
detection limit	[pmol]	0.0049	0.12	0.0039	0.0074	0.0002

†below detection limit

Like in the experiments with cell-free crude extracts, hydride generation of arsenic, selenium and to a small extent of antimony was detected (Fig. 3.8). The spectrum of methyl and hydride derivatives detected by the headspace analyses were similar to the spectrum found in the cell-free crude extract experiments (compare Fig. 3.1 and Fig. 3.8).

Assays were also repeated without HS-CoM to test whether HS-CoM is mandatory for the observed metal(loid) methylation and hydride generation. No formation of volatile metal(loid) products was detected without HS-CoM addition, indicating its indispensable role in the analyzed metal(loid) methylation and hydride generation reactions. Apparently, multi-metal(loid) methylation and hydride generation is linked

to a key reaction of methylotrophic methanogenesis, the CH_3Cob(III) dependent methylation of HS-CoM. This finding confirms the assumption of a close connection between methanogenesis and metal(loid) methylation and hydride generation.

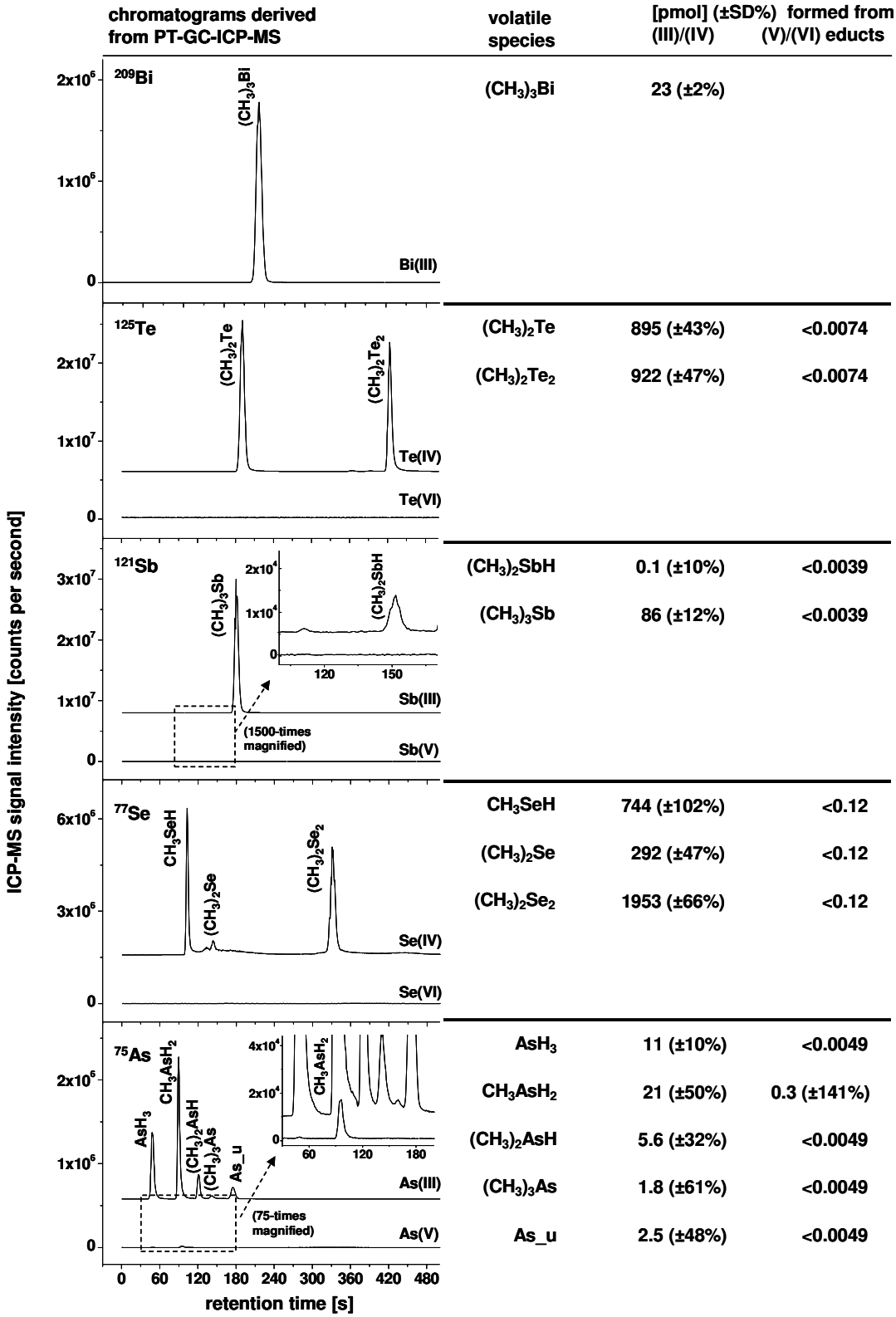

Fig. 3.8: Analyses of metal(loid) methylation and hydride generation by MtaA containing *in vitro* assays, considering the oxidation state of the applied metal(loid)s.
Headspace PT-GC-ICP-MS chromatograms of reaction mixtures containing 1 µmol CH_3Cob(III) and HS-CoM, 0.1 nmol Bi(III), Sb(III), As(III) or 10 nmol Te(IV), Te(VI), Sb(V), Se(IV), Se(VI) or As(V) (analyzed separately) and 1 mL 50 mM HEPES pH 7 are shown. Reaction was started by addition of 10 µg purified recombinant MtaA (MM_1070) to the reaction mixture followed by incubation at 30 °C for 10 min. Numerical values are derived from three independent experiments. Relative standard deviations (%SD) are in brackets. As_u: unknown volatile arsenical.

3.2. Mechanistic studies on metal(loid) methylation and hydride generation

3.2.1. Methylation and hydride generation of metal(loid)s applied in different oxidation states

In order to distinguish between different reaction mechanisms - i.e. between a oxidative methylation by a methyl carbocation and a non-oxidative methylation by a methyl carbanion or radical intermediates (Ridley et al. 1977b), respectively - methylation experiments with metal(loid) species in different oxidation states were conducted. As a “Challenger-type mechanism” requires oxidative methylation and subsequent reduction of the higher oxidized intermediates (refer to section 1.1.3.1) the reduction activity of the methylation assay was tested.

Therefore, the analyses of metal(loid) methylation and hydride generation by MtaA containing *in vitro* assays were repeated under the same conditions as in the former experiments (see section 3.1.4) but this time with metal(loid) reactants applied in their highest possible oxidation state (excluding bismuth for which only stable trivalent compounds in aqueous solutions exist). Only traces of CH_3AsH_2, formed as the sole volatile arsenical, were detected upon addition of pentavalent KH_2AsO_4 to the assays (Fig. 3.8). The amount of CH_3AsH_2 was >100-times lower relative to experiments conducted with arsenite ($AsNaO_2$). Neither methyl nor hydride derivatives of the other metal(loid)s applied in a more oxidized state as in the former experiments were found in the headspace of the *in vitro* assay. From this data it can be concluded that the *in vitro* assays are not capable to reduce pentavalent arsenic and antimony as well as hexavalent selenium and tellurium compounds to enable formation of volatile methyl and hydride metal(loid) derivatives.

3.2.2. Analyses of arsenic derivatives in solution formed by *in vitro* assays

In order to test whether MtaA, $CH_3Cob(III)$ and HS-CoM containing *in vitro* assays methylate metal(loid)s applied in their highest oxidation state without forming volatile derivatives, metal(loid) methylation was analyzed in solutions containing trivalent arsenite ($AsNaO_2$) and methyl-iodoarsine (CH_3AsI_2) as well as pentavalent methylarsonic acid ($CH_3AsO(ONa)_2$) and dimethylarsinic acid ($(CH_3)_2AsO(OH)$). The former two arsenicals represent reactants of a Challenger-like oxidative methylation

reaction and the latter two compounds the resulting first intermediates, respectively. The two pentavalent arsenicals are substrates for a reduction step according to the "Challenger Mechanism" (refer to section 1.1.3.1). Aliquots of the *in vitro* assays were subjected to hydride generation using sodium tetrahydroborate in order to convert the non-volatile arsenicals into volatile derivatives prior to PT-GC-ICP-MS analyses to detect non-volatile methylated arsenicals formed.

Arsenical analytes must be completely protonated to allow the nucleophilic hydroborate attack (Kumar and Riyazuddin 2007). As the protonation is highly dependent on the pH value of the reaction mixture, hydride generation at different pH values can be used to distinguish between pentavalent arsenicals ($AsO(OH)_3$ pK_a: 2.3; $CH_3AsO(OH)_2$ pK_a: 2.6 and $(CH_3)_2AsO(OH)$ pK_a: 6.2) and trivalent arsenicals (pK_a: >9.2) (Morin et al. 1992; Howard 1997; Kumar and Riyazuddin 2007). Only trivalent arsenic species form volatile hydrides at neutral to slightly alkalic pH. The lowering of the pH to acidic conditions allows hydride generation of pentavalent arsenic species. The efficiency of the hydride generation method used was about 90% as determined by Oliver Würfel (personal communication, PhD thesis Oliver Würfel, in preparation). The abiotic methylation and hydride generation of arsenicals by $CH_3Cob(III)$ and HS-CoM in the absence of MtaA was negligible (Tab. 3.3).

Tab. 3.3: Analyses of abiotic volatilization and methylation of trivalent and pentavalent arsenic reactants by $CH_3Cob(III)$ and HS-CoM.
The formation of volatile and non-volatile methylated As derivatives from tri- and pentavalent arsenic reactants by *in vitro* assays containing 1 µmol $CH_3Cob(III)$ and HS-CoM and 1 mL 50 mM HEPES pH 7 was investigated. Volatile species were analyzed by headspace PT-GC-ICP-MS and non-volatile species by HG-PT-GC-ICP-MS. Chemical hydride generation using sodium borhydride was separately performed at neutral pH (pH 7 – 8) and during pH gradient by continuous addition of 1 M HCl in order to differentiate between trivalent and pentavalent arsenic derivatives. Reduced and methylated products are in boldface, reactants not transformed in the in the abiotic *in vitro* assays are in italics. Blank values derived from chemical hydride generation without addition of *in vitro* assays were subtracted. Experiments were performed at least in triplicates. For calculation of total volatilization, all volatile species detected for each experiment were summarized first (data not shown). Means and relative standard deviations were calculated from these summarized values.

		$AsNaO_2$	$CH_3AsO(ONa)_2$	CH_3AsI_2	$(CH_3)_2AsO(OH)$
	reactants added [pmol] (± %SD)*	9 304 (± 2%)	57 636 (± 5%)	877 (± 2%)	1 616 (± 2%)
methylation and hydrogenation by *in vitro* assays without enzyme					
total volatilized	[pmol] (± %SD)	<DL	<DL	<DL	<DL
	[%]	<0.01	<0.01	<0.01	<0.01
total methylation‡	[pmol] (± %SD)	**61 (±17%)**	**0.37 (±13%)**	**26 (±38%)**	**10 (±62%)**
	[%]	**0.65**	**<0.01**	**2.96**	**0.62**
recovery	[%]	110	95	109	108
amounts of methylated and hydrogenated arsenic derivatives as detected by headspace and HG-PT-GC/ICP-MS analyses [pmol] (±%SD)					
trivalent volatile species	AsH_3	<DL	<DL	<DL	<DL
	CH_3AsH_2	<DL	<DL	<DL	<DL
	$(CH_3)_2AsH$	<DL	<DL	<DL	<DL
	$(CH_3)_3As$	<DL	<DL	<DL	<DL
trivalent non-volatile species	$As(OH)_3$	*9 917 (±3%)*	64 (±9%)	6.5 (±10%)	5.4 (±45%)
	$CH_3As(OH)_2$	**38 (±32%)**	110 (±15%)	*793 (±7%)*	2.5 (±52%)
	$(CH_3)_2As(OH)$	<DL	**0.37 (±13%)**	**19 (±31%)**	8.4 (±14%)
pentavalent non-volatile species	$AsO(OH)_3$	275 (±25%)	158 (±31%)	58 (±10%)	78 (±45%)
	$CH_3AsO(OH)_2$	**14 (±15%)**	*54 639 (±3%)*	44 (±71%)	10 (±24%)
	$(CH_3)_2AsO(OH)$	**7.6 (±22%)**	<DL	**8.3 (±30%)**	*1 628 (±9%)*
	$(CH_3)_3AsO$	**1.9 (±15%)**	<DL	**1.6 (±27%)**	**8.8 (±75%)**

<DL: below detection limit

*Amounts of educts added based on the concentrations of reactants determined by HG-PT-GC/ICP-MS.

‡Only derivatives with a higher methylation grade than the added reactants were considered.

The methylation as well as hydride generation of trivalent arsenicals by *in vitro* assays containing MtaA was >100-times more efficient than of pentavalent arsenic reactants (Fig. 3.9). The numeric values are given in the appendix (Tab. 10.2). As the pentavalent reactants were almost completely recovered from the *in vitro* assay without being methylated or reduced, the assays are apparently neither capable to

reduce pentavalent arsenic intermediates into trivalent derivatives nor to transfer a methyl group to pentavalent arsenicals in a non-oxidative reaction (e.g. by a methyl carbanion). The data also demonstrate that 85-96% of the methyl and hydride arsenic species formed from trivalent arsenic reactants were not oxidized in the course of their methylation. The small percentages of pentavalent arsenicals derived from experiments performed with trivalent reactants are likely formed by auto-oxidation. As pentavalent arsenicals are not methylated or reduced by the analyzed *in vitro* assays, these pentavalent arsenicals must be regarded as metabolic end products rather than intermediates of an oxidative methylation mechanism. Taken together, the data derived from experiments performed with arsenic as a representative of Group 15 elements contradict an oxidative metal(loid) methylation by the transfer of methyl carbocation at least for Group 15 elements As, Sb and Bi.

Unfortunately, the pH dependent hydride generation using sodium tetrahydroborate is not applicable to analyze the oxidation state of selenium derivatives in solution as representative of Group 16 elements. This is caused by the fact that selenate is a strong acid with an estimated pK_a value of -3 (Howard 1997).

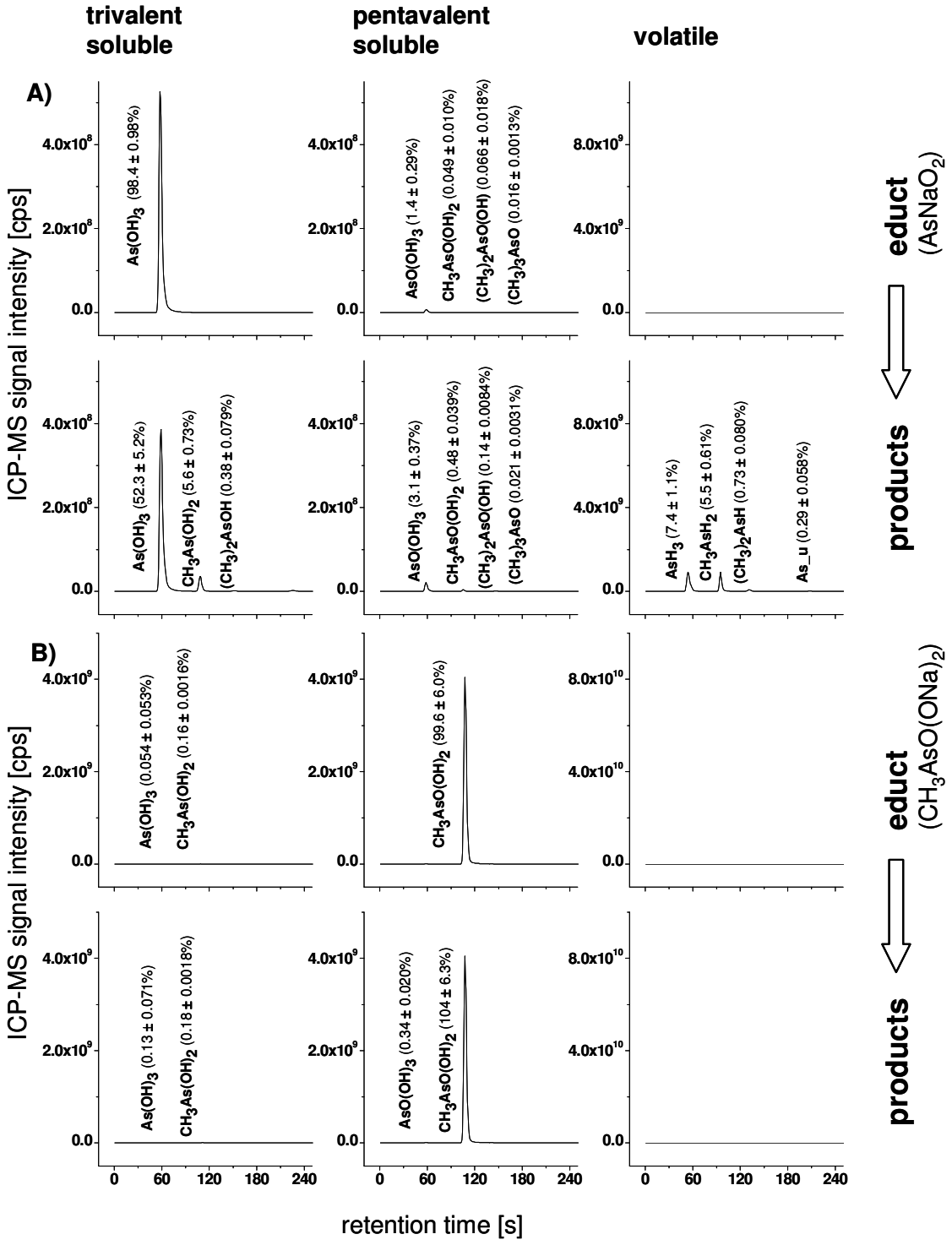

Fig. 3.9: Conversion of different arsenic reactants by MtaA containing *in vitro* assays.
PT-GC-ICP-MS chromatograms of ^{75}As signals are shown. Volatile arsenic derivatives from soluble arsenicals were formed from aliquots of reaction mixtures (1/20 of the total volume) by hydride generation at neutral or acidic pH using sodium tetrahydroborate. Volatile derivatives formed by *in vitro* assays without derivatization via sodium tetrahydroborate were derived from the headspace of the reaction vessels. *In vitro* assays contained 10 µg mL^{-1} MtaA, 1 µmol $CH_3Cob(III)$, HS-CoM, an arsenic reactant and 1 mL 50 mM HEPES pH 7. The following reactants were used: 9.3 nmol $AsNaO_2$ A), 57.6 nmol $CH_3AsO(ONa)_2$ B), 0.9 nmol CH_3AsI_2 C) and 1.6 nmol $(CH_3)_2AsO(OH)$ D). The sums of arsenicals derived from the reactants were set to 100%. The relative amount of arsenicals derived from the *in vitro* assay based on the amount of reactants added. Relative amounts from three experiments are in brackets. As_u: unknown volatile arsenical.

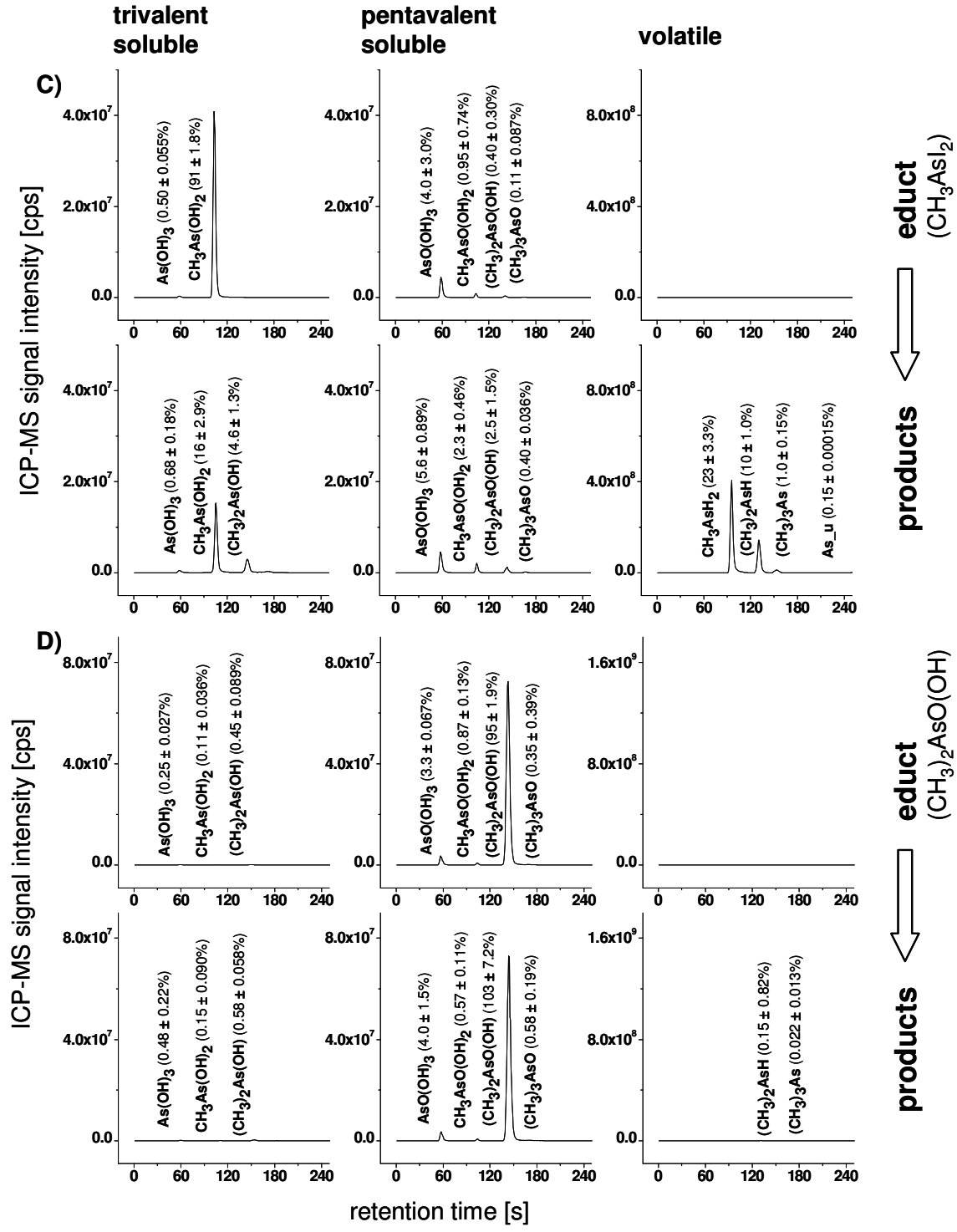

Fig. 3.9: Conversion of different arsenic reactants by MtaA containing *in vitro* assays (continued).

3.2.3. Consideration of methyl arsenic compounds with high boiling points

The balance between total amounts of arsenic reactants added and the total amounts of arsenic derivatives recovered varies in the experiments described in

section 3.2.2 (compare Tab. 10.2 and Tab. 3.3). While the recovery from the experiments with pentavalent arsenicals or in the absence of MtaA is between 95 - 110%, only 69 - 76% of total arsenic compounds were recovered from the experiments with trivalent arsenic reactants. The lower recovery of total arsenic added as trivalent derivatives from MtaA containing assays could be caused by the formation of low-volatile methyl and hydride arsenic derivatives or derivatives not susceptible to hydride generation. In addition to that, the used packed GC-column is not well suited to analyze low-volatile compounds especially in terms of quantification. However, formation of volatile arsenic derivatives with boiling points slightly higher than 100 °C from CH_3AsI_2 was observed by this technique (Fig. 3.10A). The peak of the ^{75}As ICP-MS signal appearing 153.5 s after start of the chromatography run corresponds to $(CH_3)_3As$ with a boiling point of 51 °C. The small peak appearing after 251.3 s corresponds to an arsenic derivative with a boiling point of 101 °C and the peak at 276.9 s corresponds to an arsenic derivative with a boiling point of 110 °C. The latter derivative could be assigned to $(CH_3)_2AsSH$ due to its boiling point (compare (Diaz-Bone et al. 2009)). In the absence of MtaA no derivatives with boiling points >100 °C were detected (Fig. 3.10B).

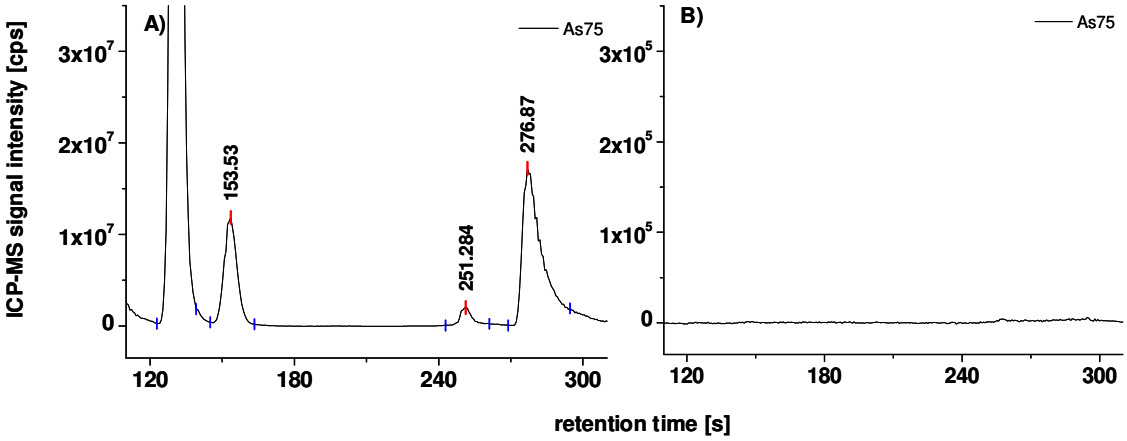

Fig. 3.10: Formation of volatile arsenic derivatives with boiling points >100 °C by MtaA containing *in vitro* assays.
Excerpts of PT-GC-ICP-MS chromatograms of the ^{75}As signal of headspace analyses of *in vitro* assays containing 1 µmol $CH_3Cob(III)$ and HS-CoM, 877 pmol CH_3AsI and 1 mL 50 mM HEPES pH 7 whether with 10 µg ml^{-1} purified recombinant MtaA (MM_1070) A) or without protein B). Retention times correspond to the following boiling points: 153.53s: 51 °C ($(CH_3)_3As$), 251.284s: 101 °C and 276.87: 110 °C.

To analyze in more detail whether low-volatile methyl and hydride arsenic compounds with boiling points much higher than 100 °C are formed, a capillary column, which is more suitable for analyses of high-boiling species, was used for

cryofocussing and subsequent chromatographic separation. In order to identify potentially new arsenic compounds, an EI-MS as molecular specific detector in addition to the element specific ICP-MS was used as described by Köster and coworkers (Kosters et al. 2005). Two examples of the GC-EI-MS analyses are given in Fig. 3.11. These analyses revealed formation of arsenic derivatives not considered by the former analyses techniques. The compounds can be assigned to $(CH_3)_2As_2O$ and $(CH_3)_3As_3$ on the basis of the fragmentation pattern. The formation of these low-volatile derivatives can explain the lower recovery of arsenic by MtaA containing *in vitro* assays in the experiments presented in section 3.2.2.

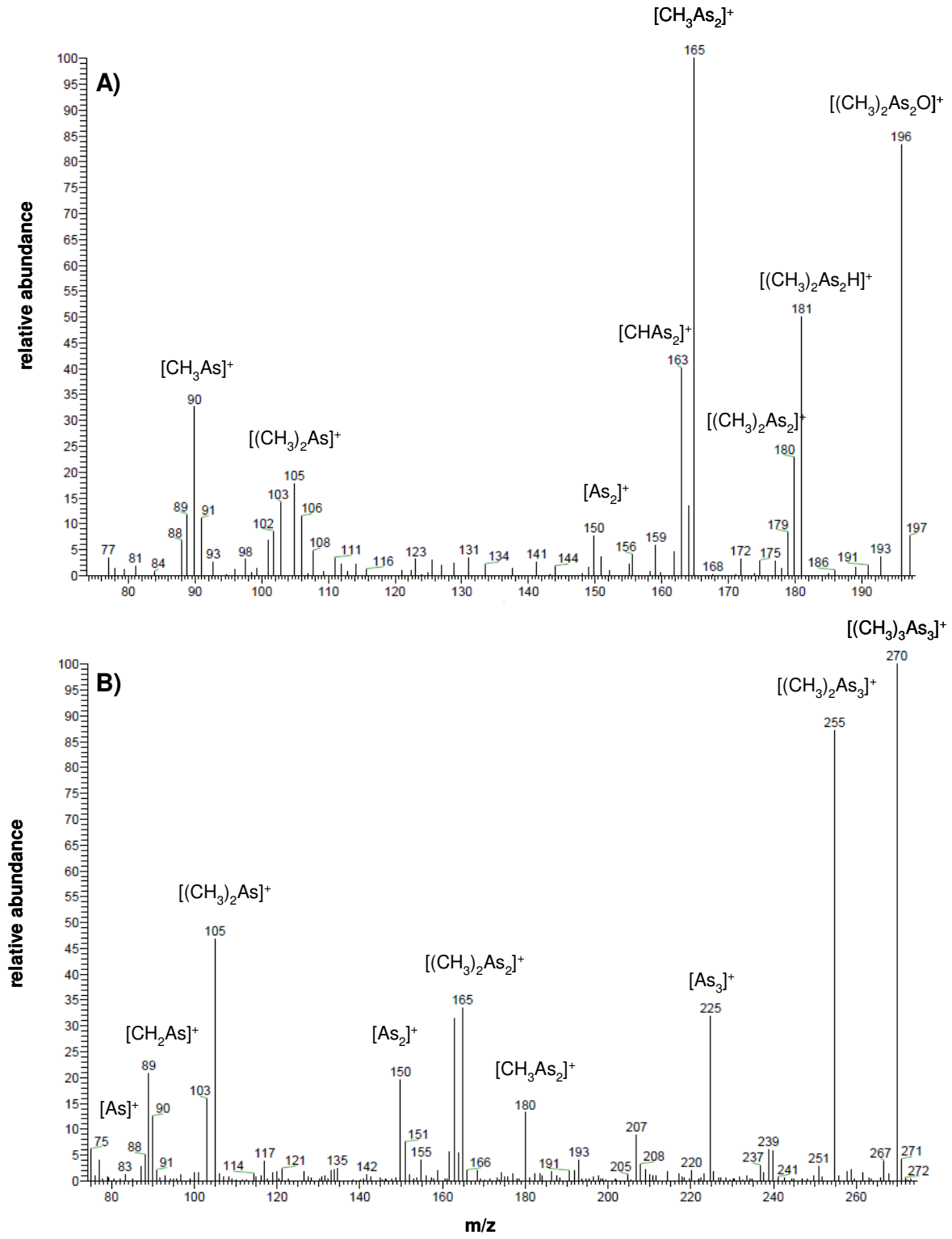

Fig. 3.11: Capillary-GC-ICP-MS/EI-MS analyses reveal formation of low-volatile arsenic derivatives by MtaA containing *in vitro* assays.
EI-MS mass fragmentograms of two low-volatile arsenic species with boiling points >150 °C as suggested from their retention times (not shown) are depicted. The volatile species were formed by *in vitro* assays containing 10 µg purified recombinant MtaA (MM_1070), 1 µmol $CH_3Cob(III)$, HS-CoM, 100 nmol CH_3AsI_2 and 1 mL 50 mM HEPES pH 7.0. The volatile species are presumably $(CH_3)_2As_2O$ A) and $(CH_3)_3As_3$ B). Analyses of EI-MS mass fragmentograms were performed by Dr. Roland A. Diaz-Bone. Data were used with kind permission of the author.

3.2.4. Analyses on the origin of hydrides forming arsine (AsH_3)

The headspace analyses of *in vitro* assays containing cell-free crude extract or MtaA showed hydride generation of arsenic, selenium and antimony. The best investigated hydride generation reaction is the one utilizing sodium tetrahydroborate (D'Ulivo 2004). Sodium tetrahydroborate transfers its hydrogen directly to the metal(loid) (Pergantis et al. 1997).

To test whether formation of arsenic hydride species observed in the *in vitro* assays performed in this thesis also originates from a hydrogenated reductant in the assay or from a reduced proton from the aqueous solution of the reaction mixtures, the *in vitro* assays were prepared either with H_2O or with heavy water (D_2O). When an *in vitro* assay containing purified recombinant 10 µg mL^{-1} MtaA, 1 mM $CH_3Cob(III)$ and HS-CoM and 0.1 mM arsenite was prepared with H_2O, the derived m/z values for arsine can be ordered to the following fragments: m/z = 75: $[As]^+$, m/z = 76: $[AsH]^+$, m/z = 77: $[AsH_2]^+$ and m/z = 78: $[AsH_3]^+$ (Fig. 3.12A). When the assays were instead prepared with D_2O to yield 58.7% relative to total H_2O, a similar mass fragment pattern but with a shift of one m/z unit for each proton carrying arsenic fragment was obtained (Fig. 3.12B). The mass fragmentograms match with those from the literature (D'Ulivo et al. 2005). The fragments with m/z values of 76, 78 and 80 are most likely caused by the 42% H_2O in the assays.

The comparison of EI-MS data of arsine formed in the presence of H_2O and in the presence of D_2O indicates formation of hydride anions by the reduction of water rather than a direct transfer of a hydride anion from a reductant like in the case of sodium tetrahydroborate.

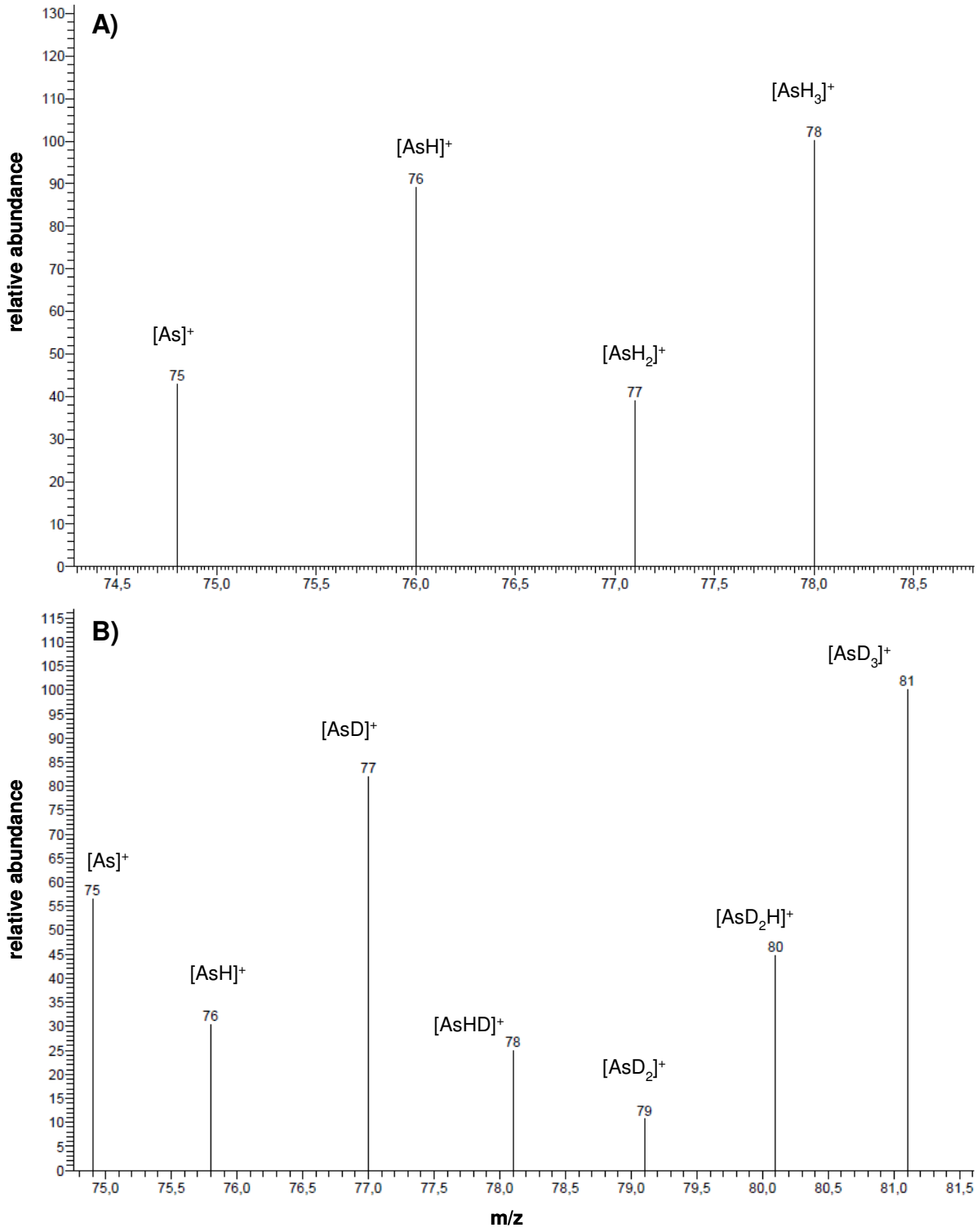

Fig. 3.12: Fragment pattern of arsine formed by MtaA containing *in vitro* assays in the presence of D_2O.
Volatile arsenic species were formed by *in vitro* assays containing 10 µg purified recombinant MtaA (MM_1070), 1 µmol $CH_3Cob(III)$ and HS-CoM, 100 nmol arsenite, 100 µL 0.5 M HEPES pH 7, 900 µl H_2O A) or a mixture of 313.1 µL H_2O and 586.9 D_2O B). Reaction mixtures were incubated at 30 °C for 10 min prior to capillary-GC-ICP-MS/EI-MS analyses. The mass fragmentograms of arsine formed in the absence of deuterium A) or in the presence of deuterium B) are depicted. The suggested elemental formulas of arsenic anion fragments corresponding to the respective m/z values are indicated. Data were used with kind permission of Dr. Roland A. Diaz-Bone.

3.2.5. UV-Vis analyses of *in vitro* assays

3.2.5.1. *Interaction of metal(loid)s with CH_3Cob(III)*

The performed *in vitro* assays contained CH_3Cob(III) as the methyl donor for metal(loid) methylation reactions. CH_3Cob(III) and its demethylated derivatives hydroxycobalamin (HOCob(III)), Cob(II) and Cob(I) exhibit specific UV-Vis spectra, allowing to distinguish between these corrinoid derivatives (Fig. 3.13). By analyzing the transformation of CH_3Cob(III) into one of its derivatives, conclusions of the nature of the transferred methyl group can be drawn. In particular, the departure of a methyl carbanion would result in the formation of HOCob(III), of a methyl radical in the formation of Cob(II) and of a methyl carbocation in the accumulation of Cob(I). For instance, the MtaA catalyzed methylation of HS-CoM *in vitro* results in the accumulation of Cob(I), thereby indicating that a methyl carbocation is transferred (LeClerc and Grahame 1996).

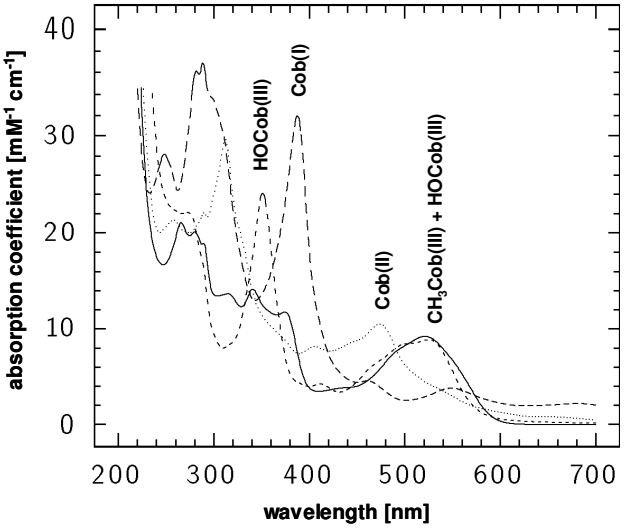

Fig. 3.13: UV-Vis spectra of different cobalamin derivatives.
The specific UV-Vis spectra of CH_3Cob(III), HOCob(III), Cob(II) and Cob(I) are indicated (Kreft and Schink 1994), modified.

Here, the absorption changes from 250-600 nm of CH_3Cob(III) were followed in *in vitro* assays verifiably capable to form methyl and hydride derivatives of numerous metal(loid)s (refer to section 3.1.4). The *in vitro* assays were performed with arsenite as representative of the Group 15 metal(loid)s arsenic, antimony and bismuth. The concentration of CH_3Cob(III) was reduced ten-fold relative to the concentration used in the former *in vitro* assays due to limitations of detection caused by the dynamic range of the UV-Vis spectrometer. The concentrations of HS-CoM and arsenite were adjusted to the CH_3Cob(III) concentration to yield the same ratios between these

compounds as in the former experiments. In one serial of experiments the concentration of arsenite was increased 10-times.

The formation of Cob(I) was evident as judged from the increase of absorption at λ = 388 nm 3 min after the reaction was started by addition of MtaA. The retention of Cob(I) accumulation might be caused by small amounts of oxygen that had to be reduced first by the evolving reductant Cob(I) prior to accumulation of the same. However, also in the presence of the higher arsenite concentration (100 nM), Cob(I) formation and accumulation was detected (Fig. 3.14).

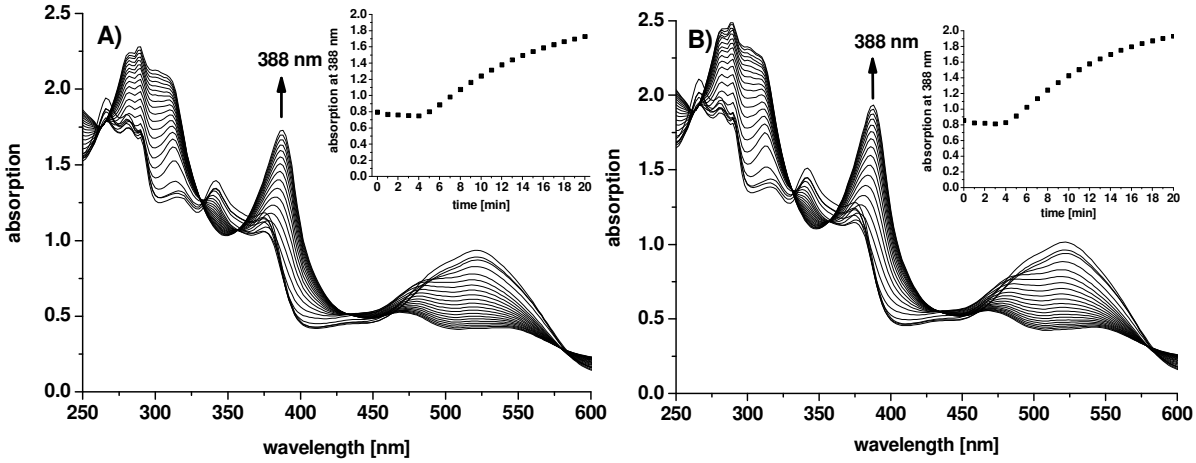

Fig. 3.14: Formation of Cob(I) by MtaA catalyzed $CH_3Cob(III)$ demethylation in the presence of As(III).
Absorption spectra of reaction mixtures containing 0.1 mM $CH_3Cob(III)$ and HS-CoM, 10 µg mL^{-1} recombinant MtaA (MM_1070), without addition of As(III) A) and in the presence of 100 nM As(III) B) were measured in 1 min intervals. Reaction mixtures were buffered by 50 mM HEPES pH 7.0. The time courses of absorption at 388 nm (Cob(I)) are depicted in the insets.

The conversion of $CH_3Cob(III)$ to Cob(I) indicates that the methyl group is transferred as a carbocation. As the methylation of metal(loid)s by carbocations was already excluded at least for the Group 15 elements As, Sb and Bi (see section 3.2.1 and 3.2.3), the accumulation of Cob(I) results most likely from the HS-CoM methylation.

As stated in the former section 3.2.2, the used HG-PT-GC-ICP-MS analytic is not suited to analyze methylation reactions of selenium in solution with also considering the oxidation state of selenium. As selenium is chemically similar to sulfur and can substitute sulfur in amino acids like cysteine etc., it was tested whether MtaA catalyzes transfers of a methyl group from $CH_3Cob(III)$ to selenium in the absence of HS-CoM. The UV-Vis spectra are thereby also valuable to analyze whether the methyl group is transferred as a carbocation, radical or anion if transferred at all. The

assays were performed with a 2.5 fold excess of selenite relative to $CH_3Cob(III)$ in the presence of 10 µg mL^{-1} MtaA or without protein (Fig. 3.15). The UV-Vis measurements followed for 30 min showed no change in the absorption spectrum of $CH_3Cob(III)$ neither with nor without MtaA. Thus, a direct methylation of selenite by $CH_3Cob(III)$ without additional cofactors can be excluded under physiological conditions and underpins thereby the requirement of HS-CoM.

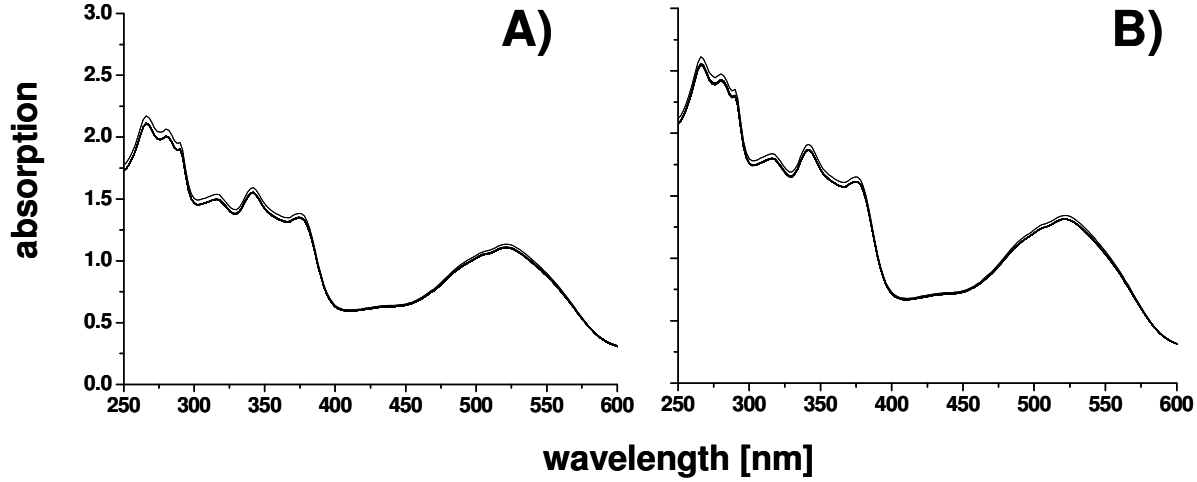

Fig. 3.15: UV-Vis analyses of $CH_3Cob(III)$ in the presence of selenite.
Absorption spectra of reaction mixtures containing 0.1 mM $CH_3Cob(III)$, 0.25 mM selenite ($NaSeO_3$) and 10 µg mL^{-1} recombinant MtaA (MM_1070) A) or no protein B) at 30 °C are depicted. Reaction mixtures were buffered by 50 mM HEPES pH 7.0. UV-Vis spectrum was measured in 1 min intervals over 30 min.

3.2.5.2. *Interaction of arsenite and arsenate with Cob(I)*

It is feasible that Cob(I) is required as an electron donor ($E'_{0\ Cob(I)/Cob(II)}$: -610 mV (Kreft and Schink 1994)) to enable the transfer of a methyl carbanion from $CH_3Cob(III)$ to a metal(loid) and thereby facilitating the observed metal(loid) methylation. This could also explain the indispensable role of HS-CoM in the *in vitro* assays. Its presence is mandatory for the MtaA catalyzed $CH_3Cob(III)$ demethylation whereupon Cob(I) is accumulated (see Fig. 3.14).

To test this hypothesis, an *in vitro* assay was first allowed to react in the absence of arsenic until $CH_3Cob(III)$ was quantitatively converted into Cob(I) as judged from unvaried UV-Vis spectra. Then, 1 µM arsenite (10% relative to the initial $CH_3Cob(III)$ concentration) was added, resulting in the rapid oxidation of Cob(I) to Cob(II) (Fig. 3.16A and B). In contrast, upon addition of 1 µM arsenate (10% relative to the initial $CH_3Cob(III)$ concentration), a dilution of the formed Cob(I) but no Cob(II) formation was observed (Fig. 3.16C and D). The oxidation of Cob(I) to Cob(II) upon arsenite

addition indicates involvement of Cob(I) in metal(loid) methylation. Cob(I) may also play a role in hydride generation. That the addition of arsenate does not provoke oxidation of Cob(I) is in accordance to the finding that the analyzed multi-metal(loid) methylation and hydride generation system is not capable to reduce or methylate pentavalent arsenicals (see section 3.2.1 and 3.2.2).

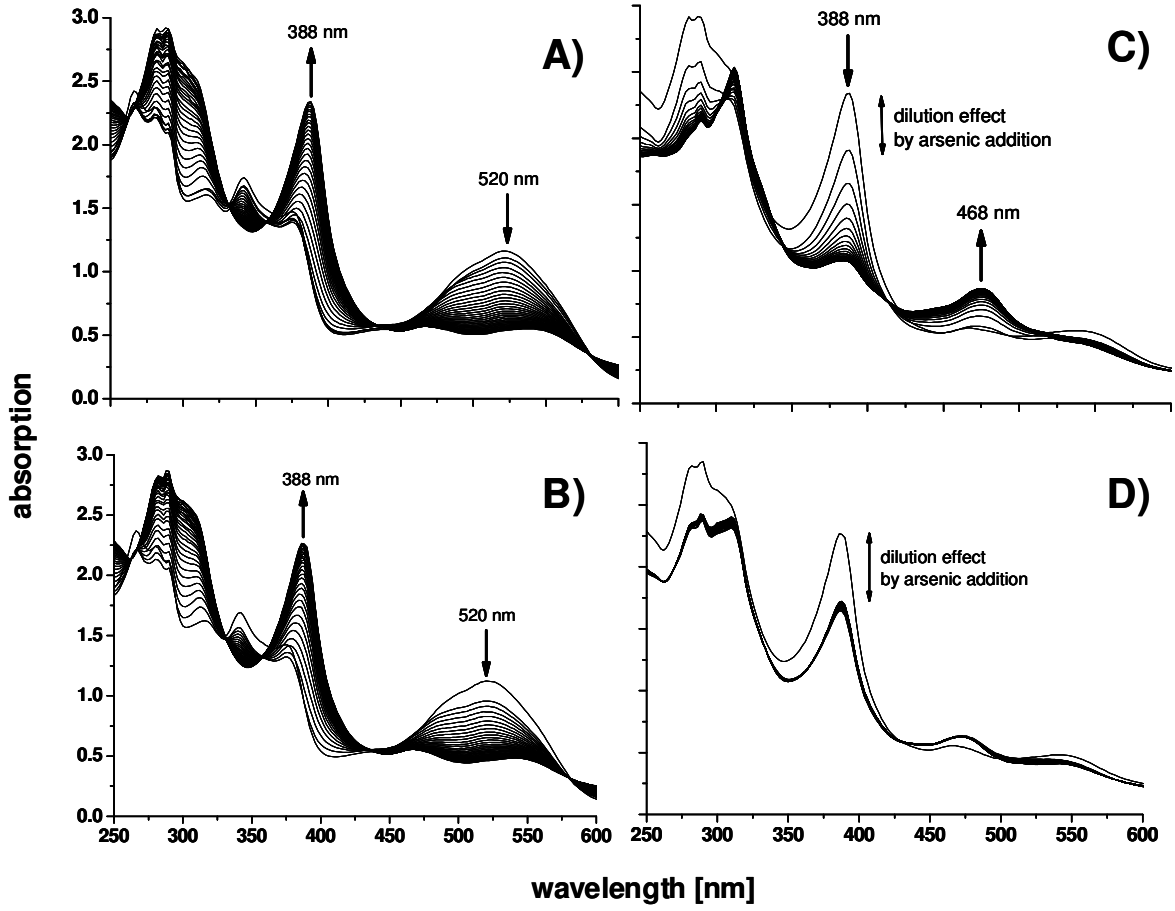

Fig. 3.16: Analyses of Cob(I), oxidized upon addition of high concentrations of arsenite but not arsenate.
Cob(I) was formed *in vitro* by MtaA (MM_1070) catalyzed demethylation of 100 nmol $CH_3Cob(III)$ in the presence of 100 nmol HS-CoM in 1mL 50 mM HEPES pH 7 at 30 °C A) and B). Upon subsequent addition of 10 nmol arsenite, oxidation of Cob(I) to Cob(II) was observed by the decrease of absorption at 388 nm and increase of absorption at 468 nm C). No oxidation of Cob(I) but only its dilution was observed when 10 nmol arsenate was added D). UV-Vis spectra from 250-600 nm were measured in 1 min intervals. Typical wavelengths: 520 nm: $CH_3Cob(III)$, 468 nm: Cob(II), 388 nm: Cob(I).

3.3. Growth of *M. mazei* at different bismuth concentrations

The effect of different bismuth concentrations on the growth of *M. mazei* was evaluated to determine the range of bismuth concentration not significantly impairing growth. Bismuth concentration at which no significant growth retardation was observed should be used for the microarray analyses to ensure preparation of sufficient amounts of intact total RNA. It can be assumed that rapid mRNA degradation comes along with increased cell degradation.

The optical density of growing *M. mazei* cultures exposed to 0-100 µM bismuth is depicted in Fig. 3.17. No significant growth retardation relative to control cultures (not exposed to bismuth) was evident at bismuth concentrations up to 10 µM. In contrast, only 24h after exposure to 100 µM bismuth no further increase of cell turbidity was detected, indicating bismuth caused cell damage not compensable by the cell.

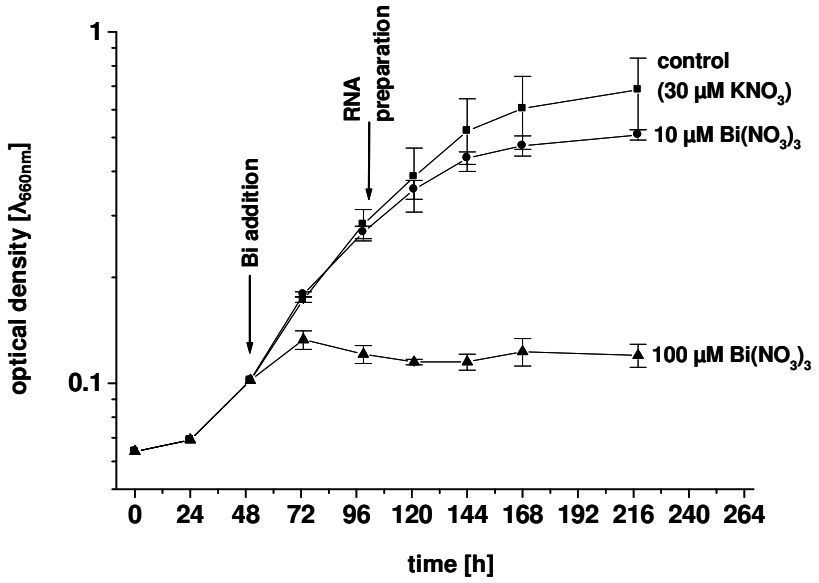

Fig. 3.17: Growth of *M. mazei* in the presence of up to 100 µM bismuth.
Increase of optical density at λ = 660 nm during growth of *M. mazei* was determined. A pre-culture (0.6 L) was propagated for 48 h and was then divided into twelve sub-cultures (0.05 L) which were supplied with $Bi(NO_3)_3$ (10-100 µM) or KNO_3 (30 µM; control cultures), respectively (first arrow). After 96 h of cultivation, one culture supplied with $Bi(NO_3)_3$ (10 µM) and one supplied with KNO_3 (30 µM) was harvest in order to isolate total RNA for further investigations (second arrow). Mean values were calculated from three cultures for each condition. Error bars indicate standard deviations.

3.4. Formation of non-volatile methylated bismuth derivatives by *M. mazei*

Almost all methanoarchaea tested are capable to form permethylated $(CH_3)_3Bi$ (Meyer et al. 2008). *M. mazei* shows one of the highest $(CH_3)_3Bi$ production rates (Thomas 2006). To date, only a few reports are available for the detection of non-volatile partly methylated bismuth derivatives (Feldmann et al. 1999; Hollmann et al. 2010). However, it is necessary to determine the amount of all methylated bismuth derivatives - volatile permethylated and non-volatile partly methylated bismuth derivatives - to estimate the metabolic cost of bismuth methylation for *M. mazei*.

Here, the amounts of non-volatile bismuth compounds formed within 48 h by *M. mazei* cultures exposed to 10 and 100 µM $Bi(NO_3)_3$ were analyzed by chemical ethyl derivatization reactions coupled to subsequent PT-GC-ICP-MS as described previously (Hollmann et al. 2010). The cultures tested reached an optical density of OD_{660}: 0.298±0.004 and 0.096±0.006 in the presence of 10 µM and 100 µM $Bi(NO_3)_3$, respectively. A typical chromatogram of an analysis is depicted in Fig. 3.18. The three distinctive peaks shown can be assigned to dimethyl monoethyl bismuth ($(CH_3)_2Bi(C_2H_5)$), monomethyl diethyl bismuth ($(CH_3)Bi(C_2H_5)_2$) and triethyl bismuth ($Bi(C_2H_5)_3$) in order of their chromatographic appearance. The prevailing perethylated bismuth derivative is converted from the inorganic $Bi(NO_3)_3$ added to the cultures and represents the portion of inorganic bismuth derivatives not methylated by *M. mazei*. Similar chromatograms were derived from all analyses of *M. mazei* cultures exposed to 10 and 100 µM $Bi(NO_3)_3$, leading to the assumption that partly methylated, non-volatile bismuth derivatives occurs in all *M. mazei* cultures exposed to bismuth.

To determine the ethylation efficiency, an inorganic Bi standard (47.8 pmol) was diluted in either 4 mL phosphate buffer pH 7 or samples prepared from *M. mazei* cultures (standard addition) prior to ethyl derivatization. The recovery of the standard from the matrix adjusted samples was low (18.8-28%) and even lower from phosphate buffer (2.6 – 13.3%). However, the poor recovery is in accordance to the reports of Hollmann and colleagues (Hollmann et al. 2010). From these data it can be assumed that the actual amount of partly methylated bismuth compounds formed by *M. mazei* is at least four-times higher than detected. Considering the determined ethylation efficiency, at least 30 nmol or 80 nmol non-volatile methylated bismuth derivatives are formed in 50 ml *M. mazei* cultures grown for 48 h in the presence of

10 µM or 100 µM $Bi(NO_3)_3$, respectively. 91–96% of the non-volatile methylated bismuth derivatives formed can be assigned to monomethylbismuth and the remaining 9–4% to dimethylbismuth.

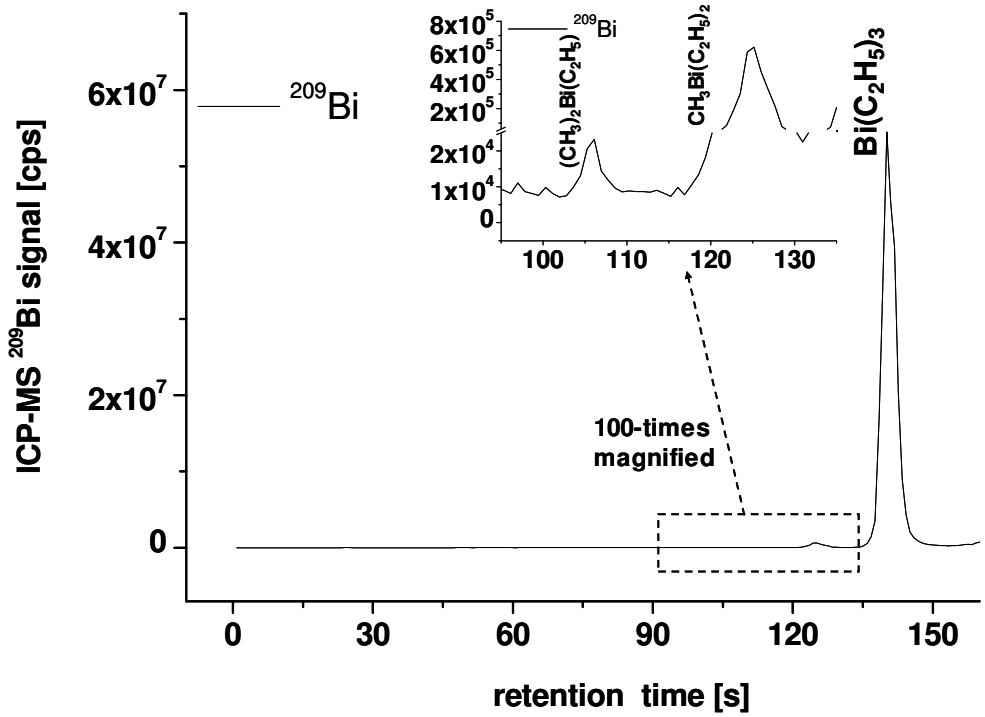

Fig. 3.18: Chromatogram of a typical PT-GC-ICP-MS analysis of chemically ethylated bismuth derivatives from *M. mazei* cultures exposed to bismuth.
The PT-GC-ICP-MS chromatogram of the ^{209}Bi signal derived from headspace analysis of a *M. mazei* culture incubated with 10 µM $Bi(NO_3)_3$ for 48 h subjected to chemical ethyl derivatization using sodium tetraethylborate is shown. Inset shows the 100-times magnified ^{209}Bi signal.

3.5. Transcriptome analyses of *M. mazei* exposed to bismuth and arsenic

The performed biochemical studies demonstrate that multi-metal(loid) methylation and hydride generation can arise from a typical methylotrophic transmethylation reaction *in vitro*. Whole genome microarray analyzes of *M. mazei* exposed to elevated bismuth and arsenic concentrations were performed to investigate whether this reaction is solely responsible for the observed multi-metal(loid) methylation and hydride generation or rather alternative pathways are involved *in vivo*. The microarray experiments were validated by northern blot analyses. Northern blot analyses were also used to analyze the expression of the *arsR/arsM* operon MM_2242-4 in more detail, because the gene product of *arsM* from

Rhodopseudomonas palustris facilitates arsenite methylation (Qin et al. 2006; Yuan et al. 2008).

3.5.1. Microarray analyses of bismuth exposed *M. mazei*

M. mazei was exposed to 10 µM $Bi(NO_3)_3$ to determine transcriptional response to elevated bismuth concentrations. Cultures for negative controls were exposed to 30 µM KNO_3 applied in the same solvent as the $Bi(NO_3)_3$ in order to avoid differential expression of enzymes involved in the nitrogen metabolism of the cell due to different nitrate availability. Total RNA was isolated from *M. mazei* cultures after 48 hours of exposure to $Bi(NO_3)_3$ or KNO_3, respectively. The isolated total RNA was then subjected to whole genome microarray analyses. A concentration of 10 µM $Bi(NO_3)_3$ and an exposure time of 48 h was chosen as clear bismuth methylation (section 3.4 and (Thomas 2006)) but negligible growth retardation was observed at this bismuth concentration and this period of exposition (Fig. 3.17).

69% of all *orf*s represented on the microarray chip match the quality criteria defined in material and methods (refer to 2.6.5) and were thus considered. 53 *orf*s (1.6% of all *orf*s printed on the chip) showed a more than two-fold higher or lower expression in response to 10 µM bismuth relative to negative controls and are listed on the next page concomitant with the designated function of their gene products, the mean values of the relative gene expression compared to control cultures (including log2 transformed values) and the corresponding standard deviations calculated from three experiments including one dye-swap reaction (Tab. 3.4).

Tab. 3.4: Regulated *orf*s of *M. mazei* in response to 10 µM bismuth.
*Orf*s with a more than two-fold elevated or reduced transcriptional level in response to 10 µM bismuth relative to control cultures not exposed to bismuth are given. Predictions for gene products were taken from GenBank (Deppenmeier et al. 2002). Data were derived from at least three microarray experiments, including one dye swap reaction. Mean values of fluorescence intensities and log2 transformed values with the corresponding standard deviations (in parentheses) are given.

Orf No.	***Gene product (based on sequence homolgy)***	***Microarray Data Bi vs. Control [mean intensity ratios (log2 ± SD)]***
Methyltransfer to methylcobalamin (methylotrophic methanogenesis)		
MM_1074	CH_3OH:corrinoid methyltransferase MtaB	2.9 (1.5 ± 0.6)
MM_1687	$(CH_3)_2NH$ corrinoid protein	3.5 (1.8 ± 0.4)
MM_1689	$(CH_3)_3N$:corrinoid methyltransferase MttB	2.3 (1.2 ± 0.1)
MM_1690	$(CH_3)_3N$ corrinoid protein	2.2 (1.1 ± 0.2)
MM_1693	$(CH_3)_2NH$:corrinoid methyltransferase	4.5 (2.0 ± 0.9)
MM_2047	$(CH_3)_3N$ corrinoid protein	2.9 (1.5 ± 0.1)
MM_2049	$(CH_3)_3N$:corrinoid methyltransferase MttB	2.3 (1.2 ± 0.3)
MM_2051	$(CH_3)_2NH$:corrinoid methyltransferase MtbB	4.3 (2.0 ± 0.7)
MM_2052	$(CH_3)_2NH$ corrinoid protein MtbC	4.0 (1.9 ± 0.7)
MM_2961	$(CH_3)_2NH$ corrinoid protein	4.1 (2.0 ± 0.7)
MM_2962	$(CH_3)_2NH$:corrinoid methyltransferase	4.1 (2.0 ± 0.7)
MM_2963	$(CH_3)_2NH$:corrinoid methyltransferase	3.4 (1.8 ± 0.5)
MM_3334	CH_3NH_2 corrinoid protein	3.2 (1.7 ± 0.4)
Energy conservation (clevage of heterodisulfide CoM-S-S-CoB)		
MM_0387	heterodisulfide reductase, subunit HdrA	4.1 (2.0 ± 0.3)
MM_0388	heterodisulfide reductase, subunit HdrC	4.0 (1.9 ± 0.4)
MM_0389	heterodisulfide reductase, subunit HdrB	3.0 (1.5 ± 0.3)
CoM synthesis		
MM_0133	threonine synthase	2.8 (1.5 ± 0.3)
MM_0134	sulfopyruvate decarpoxylase alpha chain	2.8 (1.4 ± 0.4)
Regualtory proteins		
MM_1042	transcriptional regulator	2.9 (1.5±0.6)
MM_1075	putative regulatory protein	3.7 (1.8±0.6)
MM_2242	ArsR family transcriptional regulator	2.9 (1.5 ± 0.4)
General stress response		
MM_0483	small heat shock protein	5.7 (2.2 ± 1.2)
MM_1236	protease HTPX	3.0 (1.5 ± 0.5)
Transporter		
MM_0703	hypothetical permease	2.9 (1.7 ± 0.1)
MM_1041	transporter	3.3 (1.6 ± 0.6)
MM_1516	type II secretion system protein	2.5 (1.3 ± 0.5)
MM_2576	ferrous iron transport protein B	4.6 (2.2 ± 0.2)
MM_2577	ferrous iron transport protein A	4.3 (2.1 ± 0.4)
Nitrogen metabolism		
MM_0964	glutamine synthetase	0.4 (-1.3 ± 0.2)
MM_3188	glutamine synthetase	0.3 (-1.9 ± 0.7)

Tab. 3.4: (continued).

Orf No.	*Gene product (based on sequence homolgy)*	***Microarray Data Bi vs. Control (mean intensity ratios [log2] ± SD)***
Miscellaneous		
MM_0496	phosphate acetyltransferase	3.9 (1.8 ± 0.9)
MM_1256	CdcH protein	4.6 (1.9 ± 1.2)
MM_1298	putative tRNA 2'phosphotransferase	2.3 (1.2 ± 0.1)
MM_2076	S-adenosylmethionine synthetase	3.6 (1.8 ± 0.7)
MM_2823	indole-3-glycerol phosphate synthase	5.2 (2.2 ± 0.9)
Conserved proteins		
MM_0077	hypothetical protein	0.3 (-2.0 ± 0.6)
MM_0090	conserved protein	2.5 (1.3 ± 0.3)
MM_0137	conserved protein	4.8 (2.0 ± 1.0)
MM_0452	hypothetical protein	0.3 (-1.7 ± 0.9)
MM_0908	conserved protein	2.8 (1.4 ± 0.3)
MM_1301	conserved protein	3.1 (1.5 ± 0.7)
MM_1303	conserved protein	5.2 (2.2 ± 0.8)
MM_1304	conserved protein	6.6 (2.7 ± 0.6)
MM_1305	conserved protein	4.2 (1.9 ± 0.7)
MM_1929	hypothetical protein	0.3 (-1.8 ± 0.7)
MM_2078	conserved protein	3.2 (1.6 ± 0.7)
MM_2401	conserved protein	2.3 (1.2 ± 0.1)
MM_2443	conserved protein	0.2 (-2.1 ± 0.4)
MM_2575	hypothetical protein	3.2 (1.6 ± 0.5)
MM_3197	hypothetical protein	0.4 (-1.2 ± 0.3)

The detected transcriptional level fold changes ranges from 6.6 fold (elevated) to 0.2 fold (reduced) relative to control cultures not exposed to bismuth. Affected *orf*s can be classified into the following categories due to their predicted gene products: i) methyl transfer from methylated compounds (methanol, mono-, di-, trimethylamine) to cob(I), ii) reductive cleavage of the heterodisulfide CoM-S-S-CoB formed in the course of methanogenesis, iii) *de novo* synthesis of HS-CoM, iv) transcriptional regulation, v) general stress response, vi) transport, vii) nitrogen fixation, viii) diverse synthesis reactions and ix) proteins without predicted functions. Reduced transcriptional levels were only found for *orf*s presumably involved in nitrogen fixation and some *orf*s encoding hypothetical or conserved proteins.

As $CH_3Cob(III)$ is apparently involved in metal(loid) methylation, the regulation of all *orf*s encoding designated methyltransferases utilizing $CH_3Cob(III)$ as substrate or as prosthetic group was examined more closely. These methyltransferases can be categorized into i) soluble methyltransferases transferring a methyl group from

$CH_3Cob(III)$ to HS-CoM in the course of methylotrophic methanogenesis, ii) membrane associated methyltransferases transferring a methyl group from CH_3-H_4SPT via a bound corrinoid to HS-CoM in the hydrogenotrophic and acetoclastic branch of methanogenesis and iii) the acetyl-CoA synthase/carbon monoxide dehydrogenase (CODH/ACS), transferring a methyl group from acetyl-CoA to H_4SPT via a protein bound corrinoid in acetoclastic methanogenesis. The transcriptional levels of *orf*s encoding these enzymes in *M. mazei* are summarized in Tab. 3.5. Almost all *orf*s encoding these methyltransferases were analyzable in the performed microarray experiments. Fold changes of transcriptional levels of these *orf*s range from 1.4 to 0.7 fold in response to bismuth relative to the negative controls. These *orfs* are hence considered as not regulated in response to bismuth.

The genome of *M. mazei* carries also two *orf*s homologous to genes encoding the recently characterized arsenite methyltransferase (ArsM) from *Rhodopseudomonas palustris* (Qin et al. 2006). No inductive effect of bismuth on the ArsM homologous *orf*s of *M. mazei* was observed (Tab. 3.5).

Tab. 3.5: Focused view on transcriptional expression of *orf*s encoding $CH_3Cob(III)$ dependent methyltransferases of *M. mazei* in response to 10 µM bismuth.
*Orf*s encoding $CH_3Cob(III)$, acetyl-CoA and H_4SPT dependent methyltransferases involved in methanogenesis as well as *orf*s encoding homologs of SAM dependent arsenite methyltransferase ArsM are listed. Predictions for gene products were taken from GenBank (Deppenmeier et al. 2002). Data were derived from at least three microarray experiments, including one dye swap reaction. Mean values of fluorescence intensities and log2 transformed values with corresponding standard deviations (in parentheses) are given.

Orf No.	***Gene product (based on sequence homolgy)***	***Microarray Data Bi vs. Control [mean intensity ratios (log2 ± SD)]***
Methyltransfer from methylcobalamin, acetly-CoA or N5-methyl-tetrahydromethanopterin		
MM_0176	$CH_3Cob(III)$:CoM methyltransferase MtaA	1.1 (0.0 ± 0.5)
MM_0505	$CH_3Cob(III)$:CoM methyltransferase MtbA	0.8 (-0.4 ± 0.7)[†]
MM_0684	CODH/ACS (alpha subunit)	1.1 (0.0 ± 0.6)
MM_0685	CODH/ACS (epsilon subunit)	1.0 (-0.1 ± 0.7)
MM_0686	CODH/ACS (beta subunit)	1.0 (0.0 ± 0.6)
MM_0688	CODH/ACS (delta subunit)	1.0 (-0.1 ± 0.6)
MM_0689	CODH/ACS (gamma subunit)	1.1 (0.1 ± 0.2)
MM_1070	$CH_3Cob(III)$:CoM methyltransferase MtaA	1.0 (-0.1 ± 0.7)
MM_1439	$CH_3Cob(III)$:CoM methyltransferase MtbA	1.2 (0.3 ± 0.4)
MM_1932	$CH_3Cob(III)$:CoM methyltransferase MtbA	1.4 (0.5 ± 0.1)
MM_2427	$CH_3Cob(III)$:CoM methyltransferase MtsA	1.3 (0.2 ± 0.7)
MM_0255	CH_3-H_4SPT:CoM methyltransferase	1.0 (-0.1 ± 0.4)
MM_0256	CH_3-H_4SPT:CoM methyltransferase	1.1 (0.1 ± 0.4)
MM_1540	CH_3-H_4SPT:CoM methyltransferase (subunit H)	0.7 (-0.5 ± 0.4)
MM_1541	CH_3-H_4SPT:CoM methyltransferase (subunit G)	1.1 (0.1 ± 0.6)
MM_1542	CH_3-H_4SPT:CoM methyltransferase (subunit F)	1.2 (0.1 ± 0.7)
MM_1543	CH_3-H_4SPT:CoM methyltransferase (subunit A)	1.2 (0.1 ± 0.7)
MM_1544	CH_3-H_4SPT:CoM methyltransferase (subunit B)	1.0 (-0.2 ± 0.9)
MM_1545	CH_3-H_4SPT:CoM methyltransferase (subunit C)	1.4 (0.3 ± 0.7)
MM_1546	CH_3-H_4SPT:CoM methyltransferase (subunit D)	0.8 (-0.3 ± 0.3)
MM_1547	CH_3-H_4SPT:CoM methyltransferase (subunit E)	1.3 (0.1 ± 0.4)
MM_2085	CODH/ACS (delta subunit)	1.0 (0.0 ± 0.4)
MM_2088	CODH/ACS (epsilon subunit)	1.2 (0.1 ± 0.7)
MM_2089	CODH/ACS (alpha subunit)	1.2 (0.2 ± 0.4)
MM_2251	CH_3-H_4SPT:CoM methyltransferase (subunit A)	0.9 (-0.2 ± 0.3)
arsenite specific methyltransferases		
MM_0661	arsenite methyltransferase ArsM	1.2 (0.2 ± 0.5)
MM_2243	arsenite methyltransferase ArsM	1.3 (0.4 ± 0.3)

[†] Data derived from only two experiments

3.5.2. Validation of microarray analyses of *M. mazei* exposed to bismuth by northern blots

In order to validate the microarray data performed with total RNA isolated from *M. mazei* cultures exposed to 10 µM $Bi(NO_3)_3$ or 30 µM KNO_3 (control) for 48 h, respectively, northern blot analyses of four selected *orfs* were performed. Two of the selected *orfs* showed significant regulation in response to 10 µM bismuth as suggested from the microarray experiments performed here (*mtbB* MM_2962: 4.4 fold up and *glnA* MM_3188: 3.5 fold down). Two additional genes were analyzed which showed no notable regulation in response to bismuth in the microarray experiments (MM_1070 and MM_2243).

The expression levels of the four analyzed genes as derived from 16S rRNA normalized northern blot analyses are compared to the expression values for these four genes detected by the performed microarray experiments (Fig. 3.19). Both techniques gave similar results, indicating a good reliability of the microarray data.

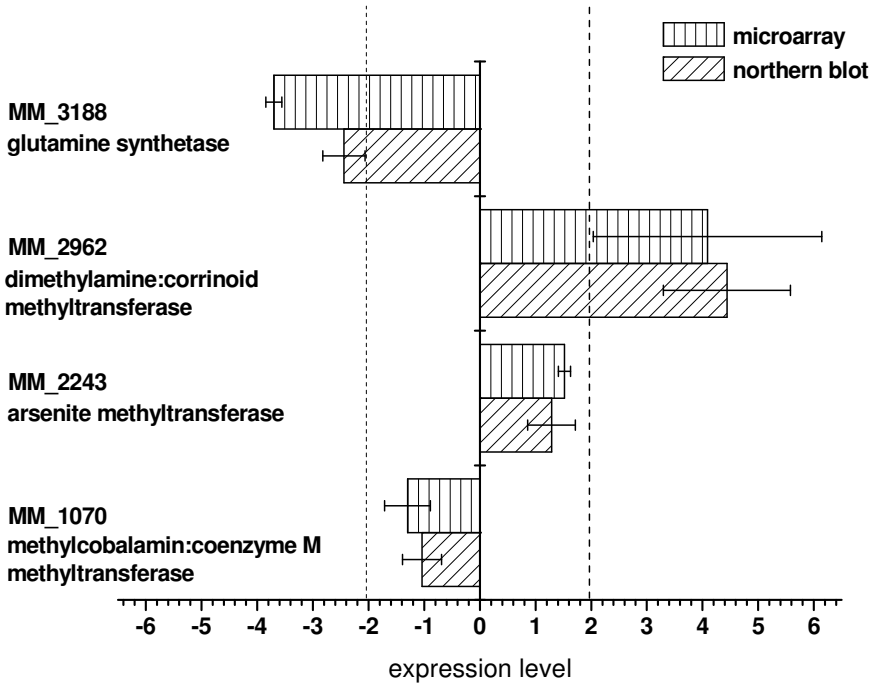

Fig. 3.19: Comparison of northern blot and microarray data derived from experiments with *M. mazei* exposed to bismuth.
Comparison between relative mRNA amounts of four *orfs* (MM_1070, MM_2243, MM_2962 and MM_3188) in response to 10 µM $Bi(NO_3)_3$ relative to control (without bismuth exposure) detected by microarrays and northern blot analyses are depicted. Total RNA was derived from three independent *M. mazei* cultures for each condition (10 µM Bi and controls) and each method, respectively.

Fig. 3.20 exemplarily shows a typical northern blot for the 16S rRNA and three out of four genes analyzed, respectively. One distinctive signal per blot was derived from northern blots hybridized with sequence specific [α-^{32}P]-CTP labeled antisense RNA probes against the 16S rRNA, MM_1070 (*mtaA*) and MM_3188 (*glnA2*), suggesting that the mRNA of these *orf*s are monocistronic. The size of these transcripts matches with their predicted size. In contrast, three distinctive signals and one weak signal were obtained upon hybridization of the northern blots with [α-^{32}P]-CTP labeled antisense RNA probes designed against MM_2962 (mtbB), pointing towards the transcription of a polycistronic mRNA. Brighter signals were detected in the lanes containing total RNA from bismuth exposed cultures than in the lanes containing total RNA from control cultures. The *orf* sizes of the corresponding northern blot signals were compared with the sizes of genes adjacent to *orf* MM_2962 in order to identify the genes of the assumed operon structure. The gene organization is depicted in Fig. 3.21A. MM_2962 and MM_2963 encode the N-terminal and the C-terminal end of the dimethylamine:corrinoid methyltransferase MtbB, respectively. The reason for using two *orf*s for *mtbB* is caused by the intragenic amber stop codon UAG, interpreted as a stop codon in the *M. mazei* genome annotation campaign. Today, it is known that this UAG codon encodes the amino acid pyrrolysine (Hao et al. 2002). The transcript sizes detected by northern blot analyses matches perfectly with the sizes of MM_2962-3 alone (1.4 kb), MM_2962_3 plus MM_2961 (2.2 kb) and MM_2962_3 plus MM_2961 plus MM_2964 (4.1 kb), thus indicating simultaneous expression of these three genes. Detection of *orf* MM_1693 by the probe designed against MM_2962 is also feasible due to the high sequence identity of both *orf*s (99%). However, in this case only the northern blot signal for the 1.4 kb and the 4.1 kb fractions could be explained (Fig. 3.20 and Fig. 3.21B). The 1.4 kb band could represent *orf* MM_1693 alone and the 4.1 kb band could correspond to a polycistronic mRNA encoded by *orf*s MM_1690 to MM_1694.

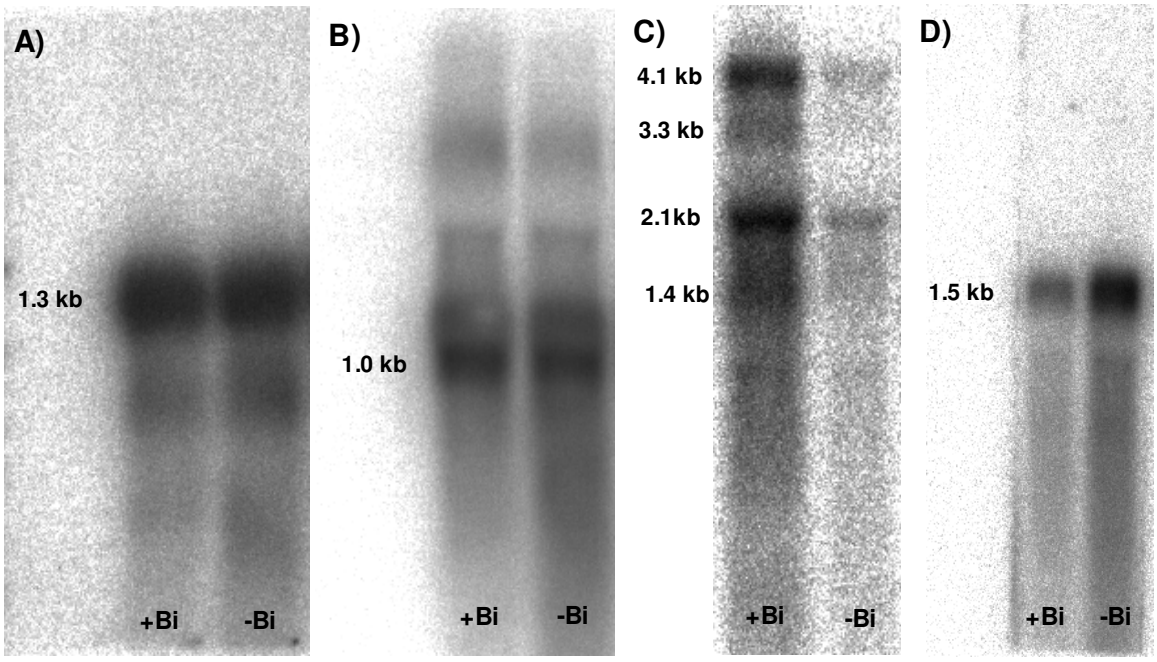

Fig. 3.20: Northern blots with specific [α-^{32}P]-CTP labeled RNA antisense probes.
Total RNA was derived from *M. mazei* cultures exposed to 10 µM $Bi(NO_3)_3$ (+Bi) or to 30 µM KNO_3 (-Bi), respectively. Probes were designed against 16S rRNA of *M. mazei* A), MM_1070 (*mtaA*) B), MM_2962 (*mtbB*) C) and MM_3188 (*glnA2*) D). Calculations of the transcript sizes based on the migration of the fragments as described in material and methods.

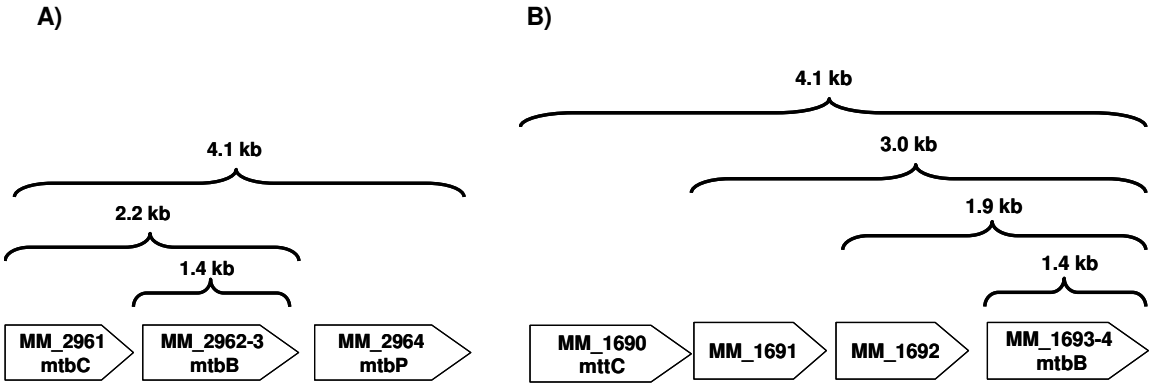

Fig. 3.21: Gene organization of MM_2962 A) and MM_1693 B).
The lengths of possible co-transcripts are depicted.

3.5.3. Preliminary microarray analyses of *M. mazei* exposed to arsenic

Arsenic is much more common in the environment than bismuth. Therefore, a preliminary microarray experiment (including a dye-swap reaction) was performed, using total RNA derived from a *M. mazei* culture exposed to 100 µM arsenate for 48 h and from a culture not exposed to arsenate as negative control. Only *orf*s were considered showing a more than two-fold higher transcriptional level in the arsenate treated culture relative to the negative control. The data are summarized in Tab. 3.6.

Only one designated transcriptional regulator from the ArsR family (MM_1257) showed more than two-fold higher expressed upon exposure to 100 µM As(V) than in the culture not treated with arsenate. Four additional *orf*s also encoding potential regulatory proteins showed elevated expression upon arsenate addition. However, none of the two annotated arsenite methyltransferase ArsM encoding *orfs* (MM_0661 and MM_2243) seem to be significantly higher expressed in response to arsenate relative to the negative control.

Tab. 3.6: Up-regulated *orf*s of *M. mazei* in response to 100 µM arsenate.
Total RNA for microarray analyses was derived from one *M. mazei* culture exposed to 100 µM KH_2AsO_4 for 48 h and from one *M. mazei* culture not exposed to arsenate, used as negative control. The obtained total RNA was subjected to two microarray experiments in order to perform a dye swap experiment. *Orf*s showing a more than two-fold higher transcript level in cells grown in the presence of arsenate relative to the negative control and not showing contrary results in the dye swap experiment were considered. *Orf*s also up-regulated in the presence of 10 µM $Bi(NO_3)_3$ were indicated in boldface.

Orf No.	***Gene product (based on sequence homolgy)***	***Microarray Data As vs. Control [mean intensity ratios]***
Methytransferases		
MM_2251	H_4SPT S-methyltransferase, subunit A	3.9
Regulatory proteins		
MM_0903	transcriptional regulator	2.5
MM_1040	predicted transcriptional regulator	4.4
MM_1094	putative transcriptional regulator	3.6
MM_1257	transcriptional regulator, ArsR family	3.6
MM_3117	transcriptional regulator, MarR family	4.4
General stress response		
MM_0483	**small heat shock protein**	**13.9**
Transporter		
MM_0736	Transporter	15.4
MM_1041	**Transporter**	**4.9**
MM_1516	**Type II secretion system protein**	**3.9**
MM_2576	**Ferrous iron transport protein B**	**7.7**
MM_2577	**Ferrous iron transport protein A**	**5.1**
MM_2578	Ferrous iron transport protein A	3.7

Tab. 3.6: (continued).

Orf No.	*Gene product (based on sequence homolgy)*	*Microarray Data As vs. Control [mean intensity ratios]*
Miscellaneous		
MM_1256	**CdcH protein**	**4.5**
MM_3118	ATP-dependent protease	22.0
Conserved proteins		
MM_0452	Hypothetical protein	2.8
MM_0737	Conserved protein	8.0
MM_0738	Conserved protein	7.1
MM_1112	Conserved protein	3.0
MM_1882	Conserved protein	3.7
MM_2245	Conserved protein	4.6
MM_2248	Conserved protein	19.7
MM_2249	Hypothetical protein	12.8
MM_2252	Conserved protein	4.2
MM_2255	Conserved protein	3.5
MM_2256	Conserved protein	4.6
MM_2258	Conserved protein	5.5
MM_2260	Hypothetical protein	8.5
MM_2261	Conserved protein	11.6
MM_2262	Conserved protein	33.0
MM_2443	Conserved protein	3.3
MM_3197	Hypothetical protein	2.1

Six *orf*s showing elevated expression upon addition of bismuth also show elevated expression in *M. mazei* exposed to 100 µM arsenate. These six *orf*s encode a potential small heat shock protein and a CdcH protein (MM_0483 and MM_1256) as well as putative transporter encoding *orf*s, including ferrous iron transport protein homologs (MM_2576-7).

Expression of *orf*s encoding designated methyltransferases utilizing $CH_3Cob(III)$ as substrate or as prosthetic group in response to arsenate is depicted separately (Tab. 3.7). An *orf* encoding a subunit of a H_4SPT dependent methyltransferase homolog (MM_2251) showed induction by 100 µM arsenate. Though, transcriptional levels for some eligible *orf*s were not obtained as these *orfs* failed the quality criteria defined in section 2.6.5.6, an overview could be drawn. The data from the microarray experiment indicate that arsenate like in the case of bismuth does not promote elevated expression of $CH_3Cob(III)$ utilizing methyltransferases involved in methanogenesis relative to cultures not exposed to the tested metal(loid)s. Only *orf* MM_2427, encoding the methyltransferase MtsA, showed a more than 2-fold

elevated expression. However, in the corresponding dye swap reaction its transcription was indifferent (data not shown). Thus, this finding is questionable.

Tab. 3.7: Focused view on transcriptional expression of *orf*s encoding $CH_3Cob(III)$ dependent methyltransferases of *M. mazei* in response to 100 µM arsenate.
*Orf*s encoding $CH_3Cob(III)$, acetyl-CoA and H_4SPT dependent methyltransferases involved in methanogenesis are listed. Predictions for gene products were taken from GenBank (Deppenmeier et al. 2002). Data were obtained from two microarray experiments including one dye swap reaction. Total RNA was isolated from a one *M. mazei* culture exposed to 100 µM arsenate and from one culture not exposed to arsenate, respectively. Mean values of fluorescence intensities are given (nd: not detected).

Orf No.	*Gene product (based on sequence homolgy)*	*Microarray Data As vs. Control [mean intensity ratios]*
Methyltransfer from methylcobalamin, acetly-CoA or N5-methyl-tetrahydromethanopterin		
MM_0176	$CH_3Cob(III)$:CoM methyltransferase MtaA	0.8
MM_0505	$CH_3Cob(III)$:CoM methyltransferase MtbA	nd
MM_0684	CODH/ACS (alpha subunit)	1.4
MM_0685	CODH/ACS (epsilon subunit)	1.4
MM_0686	CODH/ACS (beta subunit)	1.3
MM_0688	CODH/ACS (delta subunit)	0.9
MM_0689	CODH/ACS (gamma subunit)	1.1
MM_1070	$CH_3Cob(III)$:CoM methyltransferase MtaA	0.7
MM_1439	$CH_3Cob(III)$:CoM methyltransferase MtbA	0.9
MM_1932	$CH_3Cob(III)$:CoM methyltransferase MtbA	nd
MM_2427	$CH_3Cob(III)$:CoM methyltransferase MtsA	2.2*
MM_0255	CH_3-H_4SPT:CoM methyltransferase	1.1
MM_0256	CH_3-H_4SPT:CoM methyltransferase	0.9
MM_1540	CH_3-H_4SPT:CoM methyltransferase (subunit H)	nd
MM_1541	CH_3-H_4SPT:CoM methyltransferase (subunit G)	1.0
MM_1542	CH_3-H_4SPT:CoM methyltransferase (subunit F)	1.0
MM_1543	CH_3-H_4SPT:CoM methyltransferase (subunit A)	1.1
MM_1544	CH_3-H_4SPT:CoM methyltransferase (subunit B)	0.9
MM_1545	CH_3-H_4SPT:CoM methyltransferase (subunit C)	1.2
MM_1546	CH_3-H_4SPT:CoM methyltransferase (subunit D)	1.1
MM_1547	CH_3-H_4SPT:CoM methyltransferase (subunit E)	1.3
MM_2085	CODH/ACS (delta subunit)	1.4
MM_2088	CODH/ACS (epsilon subunit)	nd
MM_2089	CODH/ACS (alpha subunit)	1.0
MM_2251	CH_3-H_4SPT:CoM methyltransferase (subunit A)	3.9

*transcriptional expression ratio of dye swap experiment was below 1.8 fold

3.5.4. Expression analyses of *arsR/arsM* genes by northern blot analyses

Northern blot analyses of total RNA derived from *M. mazei* exposed to 100 µM pentavalent arsenate and 10-100 µM trivalent arsenite were performed in order to

test whether the designated *arsM* (MM_2243) and the adjacent homolog to a *arsR* repressor regulator gene (MM_2242) is regulated in response to arsenic.

Only when *M. mazei* is exposed to relative high concentrations of trivalent arsenite (≥100 µM), the *arsR/arsM* operon is regulated as suggested from the northern blot data (Fig. 3.22). A signal corresponding to a 1.2 kb transcript matching the theoretical length of the *arsR* homolog encoded by *orf* MM_2242 was derived from all samples including the negative control (prepared from cells not exposed to arsenic) upon hybridization with specific [α-^{32}P]-CTP labeled RNA antisense probes against MM_2242 (*arsR*) (Fig. 3.22A). A significant stronger expression of that transcript relative to the negative control was, however, only observed in the sample prepared from cells exposed to 100 µM $As(OH)_3$ (2-fold higher relative to negative control). At a lower arsenite concentration or in the case of 100 µM pentavalent KH_2AsO_4, the expression levels do not notably exceed the expression level of the negative control (10 µM $As(OH)_3$: 1.36-fold higher relative to negative control and 100 µM KH_2AsO_4: 1.16-fold higher relative to negative control). Upon hybridization with specific [α-^{32}P]-CTP labeled RNA antisense probes against MM_2243 (*arsM*) again only the sample prepared from the culture incubated with 100 µM $As(OH)_3$ showed notable expression of two distinct bands (with 1.2 and 1.8 kb length) and a smear at the height corresponding to 0.7 kb (Fig. 3.22B). The length of the transcripts matches perfectly with *orf*s MM_2242 to MM_2244, indicating a transcriptional unit (compare Fig. 3.23). *Orf* MM_2244 is encoding a putative arsenate reductase. Expression levels were determined to be 4.15-fold (MM_2242+MM_2243), 2.6-fold (MM_2242+MM_2243+MM_2244) and 2.19-fold (MM_2243) higher relative to the negative control, respectively.

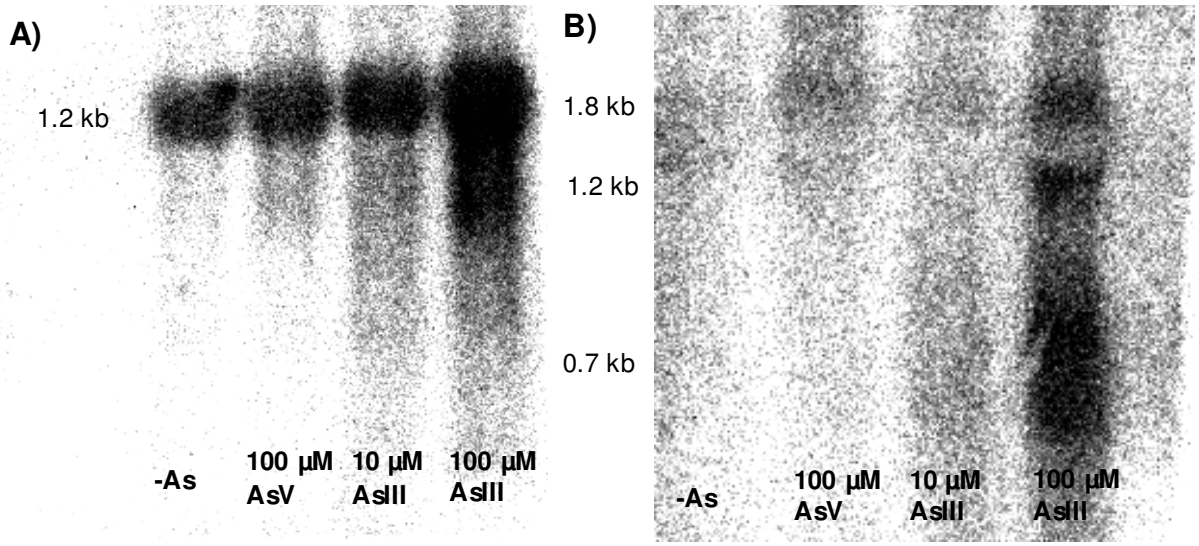

Fig. 3.22: Expression of the *arsR/arsM* operon (MM_2242-4) of *M. mazei* in responds to high arsenic concentrations.
Northern blot analyses of RNA derived from *M. mazei* exposed to 100 µM KH_2AsO_4 (AsV), and 10-100 µM $As(OH)_3$ (AsIII), respectively, are shown. RNA derived from cultures not exposed to arsenic (-As) was used as negative control. Specific [α-^{32}P]-CTP labelled RNA antisense probes were designed against MM_2242 (*arsR*) A) and MM_2243 (*arsM*) B).

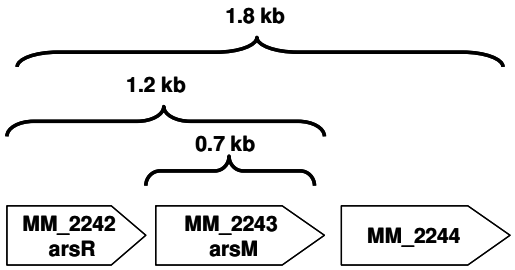

Fig. 3.23: Organization and length of *orf*s MM_2242, MM_2243 and the adjacent *orf* MM_2244.
MM_2244 presumably encodes an enzyme with arsenate reductase activity.

4. Discussion

4.1. Multi-metal(loid) methylation and hydride generation is linked to methanogenesis

4.1.1. Multi-metal(loid) methylation in *M. mazei* requires no induction by metal(loid)s

The exceptional versatility of methanoarchaea to form methyl and hydride derivatives of various metal(loid)s was reported in numerous studies (McBride and Wolfe 1971; Michalke et al. 2002; Michalke et al. 2006; Meyer et al. 2008). Some of these studies suggested $CH_3Cob(III)$ as the decisive methyl group donor for the biomethylation of arsenic and bismuth in methanoarchaea (McBride and Wolfe 1971; Michalke et al. 2002). $CH_3Cob(III)$ plays a central role in a key reaction in all methanogenic pathways, the methylation of HS-CoM (see 1.1.4.4). This important role of $CH_3Cob(III)$ in methanogenesis is reflected by the comparably high content of $CH_3Cob(III)$ and its derivatives in methanoarchaea, especially when grown on methanol (Krzycki and Zeikus 1980). The concentration of cobalamin derivatives in methanol grown *M. barkeri* is about 1.3 mM when assuming a cell water content of 3 µl mg^{-1} dry weight as suggested by Balch and Wolfe (Balch and Wolfe 1979). The high concentration of $CH_3Cob(III)$ alone can, however, not trigger the formation of methylated metal(loid) derivatives as demonstrated in this thesis. Abiotic *in vitro* assays containing 1 mM $CH_3Cob(III)$ are not capable to form volatile methyl and hydride derivatives of As, Se, Sb, Te and Bi (see section 3.1.1), though this $CH_3Cob(III)$ concentration exceeds the $CH_3Cob(III)$ concentrations used in the abiotic negative controls in the studies of McBride and Wolfe as well as Michalke and coworker 100-1000 fold (McBride and Wolfe 1971; Michalke et al. 2002).

This finding clearly supports the assumption that metal(loid) methylation and hydride generation by methanoarchaea is mediated by enzymes as suggested earlier (McBride and Wolfe 1971; Michalke et al. 2002). To test whether the required enzymes for multi-metal(loid) methylation originate from a constitutive energy metabolism pathway and thus need no previous induction, *in vitro* experiments with soluble cell-free crude extracts were performed. To avoid inductive expression of alleged metal(loid) specific methylation systems, the cell-free crude extracts were

prepared from methanol grown *M. mazei* cultures that were not exposed to elevated metal(loid) concentrations. Formation of volatile methyl arsenic and bismuth derivatives is observed when $CH_3Cob(III)$ and HS-CoM containing *in vitro* assays are amended by these cell-free crude extracts. Not only dimethylarsine ($(CH_3)_2AsH$) is derivatized as formed by cell-free crude extract prepared from *Mb. bryantii* cultures not exposed to arsenic (McBride and Wolfe 1971), but also AsH_3, CH_3AsH_2 and $(CH_3)_3As$. The *M. mazei* cell-free crude extract is also capable to form $(CH_3)_3Bi$ like *Mb. formicicum* cell-free crude extracts (Michalke et al. 2002). Additionally, it was tested whether further metal(loid)s which are converted into volatile methyl derivatives by growing *M. mazei* cultures (Meyer et al. 2008) can be converted into volatile methyl derivatives by this assay as well. The analyses clearly showed formation of volatile methyl selenium, antimony and tellurium derivatives. These findings indicate that the formation of volatile methyl metal(loid) derivatives observed for growing *M. mazei* cultures requires no previous induction of specific metal(loid) methylation systems.

In addition to the volatile permethylated metal(loid) derivatives formed by growing *M. mazei* culture, also hydride generation of arsenic, selenium and antimony was observed in the *in vitro* assays. A possible cause for the hydride generation *in vitro* will be discussed later.

4.1.2. Multi-metal(loid) methylation can be caused by a key reaction of methylotrophic methanogenesis

The metal(loid) methylation is enabled by $CH_3Cob(III)$ and enzymes constitutively expressed in methanol grown *M. mazei* cultures. The methyltransferase MtaA is presumably the most important $CH_3Cob(III)$ dependent enzyme in methanol grown *M. mazei* cells. MtaA is a soluble methyltransferase that catalyzes the formation of CH_3-S-CoM from $CH_3Cob(III)$ and HS-CoM and is thus an integral part of the methylotrophic methanogenesis from methanol in *Methanosarcina* species (Grahame 1989; Ferguson et al. 1996; Harms and Thauer 1996). Recombinant MtaA was tested for its capability to substitute for the cell-free crude extract to trigger the above described multi-metal(loid) methylation and hydride generation activity (see section 4.1.1). The purified recombinant methyltransferase MtaA (MM_1070) is clearly capable to catalyze the requested HS-CoM methylation in the presence of $CH_3Cob(III)$ and HS-CoM. Moreover, the addition of MtaA to *in vitro* assays

containing $CH_3Cob(III)$ and HS-CoM enables the formation of volatile metal(loid) derivatives. The same permethylation volatile metal(loid) derivatives are formed by *in vitro* assay as by growing *M. mazei* cultures (Meyer et al. 2008). The volatile metal(loid) derivatives also include volatile hydride species of arsenic, selenium and antimony. The pattern of volatile methyl and hydride derivatives formed in the presence of MtaA is similar to the species pattern derived from the *in vitro* assays performed with cell-free crude extracts of *M. mazei* (compare Fig. 3.1 and Fig. 3.8). It can be concluded that the observed capability of *M. mazei* to methylate numerous metal(loid)s is connected to the catalytic activity of MtaA and is hence directly linked to methanogenesis.

The central cofactor of methanogenesis, HS-CoM, was added to the *in vitro* assays in both cases, with cell-free crude extract or with purified recombinant methyltransferase MtaA. However, the *in vitro* experiments performed with cell-free crude extracts prepared from *Mb. bryantii* and *Mb. formicicum* needed no amendment of HS-CoM to enable the formation of volatile methyl derivatives of arsenic and bismuth (McBride and Wolfe 1971; Michalke et al. 2002). To test whether HS-CoM is dispensable for the metal(loid) methylation and hydride generation reaction observed, the *in vitro* assays with purified recombinant MtaA were repeated without HS-CoM. Without addition of HS-CoM, no volatile methyl and hydride derivatives of applied metal(loid)s are formed. As the $CH_3Cob(III)$ dependent methylation of HS-CoM *in vitro* results in the accumulation of the strong reductant Cob(I) as determined by UV-Vis analyses (see section 3.2.5) according to the following equation

$$CH_3Cob(III) + HS-CoM \xrightarrow{MtaA} Cob(I) + CH_3-S-CoM + H^+,$$

the accumulation of Cob(I) is presumably decisive for the observed multi-metal(loid) methylation and hydride generation. However, the highly reducing Cob(I) is kept on a low level *in vivo* (Sauer and Thauer 1999). The lower availability of this reductant might explain the lack of metal(loid) hydride generation by *M. mazei in vivo*.

But if HS-CoM is mandatory, why was methylation and hydride generation of As and Bi observed in the *in vitro* assays performed with cell-free crude extracts of *Mb. bryantii* and *Mb. formicicum* without HS-CoM amendment (McBride and Wolfe 1971; Michalke et al. 2002)? As in both studies the cell-free crude extracts were not subjected to dialyzation, it can not be excluded that sufficient HS-CoM remained in

the cell-free crude extracts to enable the metal(loid) methylation and hydride generation reactions. Balch and Wolfe determined high concentrations of HS-CoM in methanoarchaea (Balch and Wolfe 1979). According to their study *Mb. bryantii, Mb. formicicum* and methanol grown *M. barkeri* contain about 3 mM, 2.8 mM and 5.4 mM HS-CoM, respectively. As efficient metal(loid) methylation and hydride generation is observed in the presence of 1 mM HS-CoM, only one third or even less of the determined HS-CoM concentration found in the tested methanoarchaea must have been remained in the cell-free crude extracts of *Mb. bryantii* and *Mb. formicicum* to enable the reported metal(loid) methylation and hydride generation of arsenic and bismuth.

4.1.3. The mechanistic of multi-metal(loid) methylation differs from the MtaA catalyzed HS-CoM methylation

MtaA is a zinc containing protein (LeClerc and Grahame 1996) which catalyzes the HS-CoM methylation by forming a zinc-thiolate complex as previously demonstrated for MtaA and its isoenzyme MtbA from *M. barkeri*, respectively (Gencic et al. 2001; Kruer et al. 2002). This zinc-thiolate complex then demethylates $CH_3Cob(III)$, forming CH_3-S-CoM and Cob(I). The methyl group in this reaction is transferred as a carbocation as demonstrated by UV-Vis analyses of the demethylated corrinoid (LeClerc and Grahame 1996).

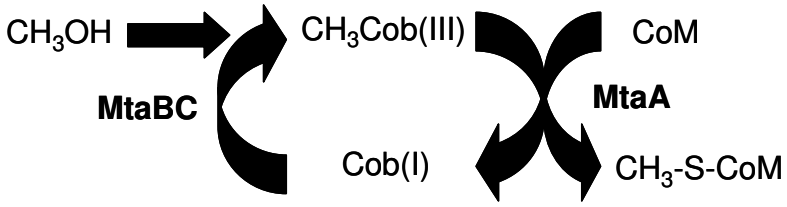

Fig. 4.1: Scheme of HS-CoM methylation in the methanol utilizing methanogenic pathway. The methyl group of methanol is cleaved off by MtaBC and transferred to the reduced cofactor Cob(I). Then, MtaA catalyzes the methyl transfer from $CH_3Cob(III)$ to HS-CoM by deprotonating the thiol group of HS-CoM (Sauer and Thauer 2000).

When assuming a corresponding mechanism for metal(loid) methylation, the transfer of a methyl carbocation to the investigated metal(loid)s would result in an oxidation of the metal(loid). Alternatively, methylation by a methyl carbanion through a nucleophilic substitution reaction would not alter the oxidation state of the metal(loid). Also a mechanism involving radical transition states is feasible. The two latter mechanisms would require external supply of electrons. Likewise, electrons are

needed for the formation of volatile hydrides of arsenic, selenium and antimony, observed during CH_3Cob(III) dependent HS-CoM methylation by MtaA. The nature of the transferred methyl group was analyzed in this thesis and is discussed in the following sections.

4.1.3.1. *The observed metal(loid) methylation of Group 15 metal(loid)s is a non-oxidative process*

To analyze whether the observed multi-metal(loid) methylation mechanism is oxidative or non-oxidative, it is adjuvant to know the common oxidation states of the metal(loid)s subjected to methylation in aqueous solutions. In the case of bismuth the oxidation states +3 and +5 are feasible. However, pentavalent bismuth compounds are not stable in aqueous solutions (Sadler et al. 1999; Michalke et al. 2002; Filella 2010). In contrast, stable trivalent and pentavalent compounds of both arsenic and antimony are common in the environment (Bentley and Chasteen 2002). The most important oxidation states of selenium and tellurium are -2, 0, +4 and +6 (Chasteen and Bentley 2003).

From the metal(loid) volatilization experiments with MtaA it is evident that inorganic As, Sb and Bi (applied as trivalent salts) as well as Se and Te (applied as tetravalent salts) are converted into volatile methyl derivatives. Here, not only monomethyl metal(loid) species but also di- and trimethyl derivatives are formed, pointing towards the involvement of more than one methylation step. If methylated oxidatively, the first methylation step would result in a higher oxidation state of the methylated metal(loid). In this case, the product from the first methylation step requires reduction prior a second transfer of a methyl carbocation as formulated in the “Challenger Mechanism” of arsenic methylation presented in section 1.1.3.1 (Challenger 1945). To test whether appropriate reduction reactions are operative in the *in vitro* assay, the same metal(loid)s were added to the assay but in their highest oxidation state (pentavalent in the case of arsenic and antimony and hexavalent in the case of selenium and tellurium). As demonstrated, the volatilization via methylation and hydride generation processes of the higher oxidized metal(loid) reactants is negligible in comparison to the more reduced metal(loid) reactants (refer to section 3.2.1). This finding indicate that pentavalent Group 15 metal(loid)s and hexavalent Group 16 metalloids are not reduced in the *in vitro* assays to form the more reduced metal(loid) compounds

efficiently volatilized by the MtaA containing *in vitro* assays. An oxidative methylation mechanism seems hence unlikely.

To confirm that the metal(loid) methylation analyzed in this thesis is not an oxidative reaction, methylation of arsenic was analyzed in solution by HG-PT-GC-ICP-MS (refer to section 3.2.2). As trivalent arsenic reactants added to the reaction mixtures are apparently not oxidized to pentavalent intermediates upon methylation but instead trivalent non-volatile reaction products of arsenic methylation were detected in solution along with the volatile methyl and hydride derivatives, the assumption of a non-oxidative methylation pathway seems likely.

Moreover, these experiments demonstrate that in the case of pentavalent arsenicals neither reduction nor methylation in solution occurs. This indicates that the accumulated Cob(I) formed by MtaA catalyzed demethylation of CH_3Cob(III) is not capable to reduce pentavalent arsenicals to trivalent arsenicals despite its negative redox potential ($E'_{0\ Cob(I)/Cob(II)}$: -610 mV (Kreft and Schink 1994)). This is also confirmed by the observation that addition of high arsenate concentration to enzymatically formed Cob(I) does not result in the oxidation of the corrinoid (Fig. 3.16), thereby excluding a redox reaction between arsenate and Cob(I). The finding is in good accordance to the studies of McBride and Wolfe also stating that the reduced demethylation product of CH_3Cob(III), Cob(I) alone, is not capable to reduce arsenate (McBride and Wolfe 1971). The lack of arsenate reduction by Cob(I) is presumably caused by the fact that arsenate (pK_a: 2.3) is in contrast to arsenite (pK_a: 9.2) deprotonated at neutral pH (Howard 1997). Thus, arsenate is almost completely negatively charged at pH 7, hindering its reduction by a nucleophile like in the case of hydride generation of arsenate by using sodium tetrahydroborate at neutral pH (Kumar and Riyazuddin 2007). A similar behavior of pentavalent antimonate (pK_a: 2.7) and trivalent antimonite (pK_a: 11) at neutral pH is likely (Andreae et al. 1981). The ionization of these pentavalent metal(loid) oxo-species at neutral pH can also explain why a non-oxidative methylation of pentavalent arsenicals does not occur as the negative charge presumably repels the attack of a methyl carbanion.

4.1.3.2. *Proposed mechanism of methylation of Group 15 metal(loid)s*

Cob(I) plays a decisive role in the transformation of arsenite into methyl and hydride derivatives. Enzymatically formed Cob(I) is oxidized to Cob(II) upon addition of

arsenite (Fig. 3.16). The highly reductive Cob(I) formed upon HS-CoM dependent CH_3Cob(III) demethylation by MtaA presumably increase the electron density at CH_3Cob(III) and thereby enabling the transfer of a methyl carbanion to a metal(loid) compound via a nucleophilic substitution reaction, provided the metal(loid) is neither negatively charged nor shielded by its functional groups.

A similar concept was introduced earlier and the carbanion termed “cryptocarbanion” (Schrauzer et al. 1973; Schrauzer 1974; Schrauzer 1977; Bentley and Chasteen 2002). It was suggested that electrons are delivered upon dithiol formation to promote the heterolytic cleavage of the Co-C bond of CH_3Cob(III), forming a methyl carbanion, Cob(I) and a disulfide. The methyl carbanion was assumed to methylate a metal(loid) in a nucleophilic substitution reaction (see section 1.1.3.2).

Considering all these data including the fact that pentavalent bismuth derivatives are unlikely in aqueous solutions, the observed methylation of Group 15 elements As, Sb and Bi in solution can be considered as a non-oxidative mechanism as exemplified on arsenic (Fig. 4.2). The scheme also includes the hydride generation steps leading to the formation of the hydrides observed in the *in vitro* assays. Here, neither methylation nor hydride generation are formally regarded as a reduction of the metal(loid)s as the electronegativities of As, Sb and Bi are lower than the electronegativities of carbon and hydrogen according to the Pauling scale used in this thesis (Tab. 1.2).

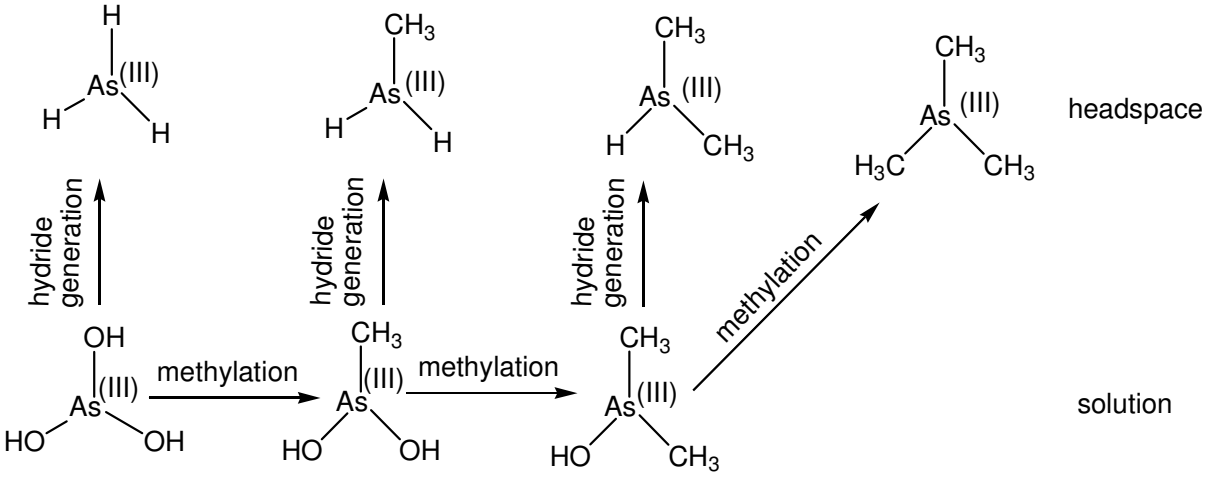

Fig. 4.2: Proposed methylation pathway of Group 15 metal(loid)s As, Sb and Bi as exemplified on arsenic.
The data derived in this thesis indicate a non-oxidative methylation of Group 15 elements As, Sb and Bi by a methyl carbanion. The observed formation of hydrides by *in vitro* assays is considered in the schema. Oxidation numbers for arsenic (in brackets) were calculated on the basis of the electronegativity according to the Pauling scale given in Tab. 1.2.

4.1.3.3. *Formation of metal(loid) hydrides* in vitro

Volatile hydride species of arsenic, selenium and antimony are formed by *in vitro* assays containing $CH_3Cob(III)$, HS-CoM and either cell-free crude extracts of *M. mazei* or purified recombinant MtaA. This finding is contrary to the observations made with growing *M. mazei* cultures (Meyer et al. 2008).

The hydride generation of arsenite requires presumably a strong reductant. However, a direct transfer from a reducing agent like in the case of sodium tetrahydroborate (Pergantis et al. 1997; D'Ulivo 2004) is unlikely as comparative analyses of arsine formed either in the presence of H_2O or in the presence of D_2O indicate that the hydride originates from the solvent (see section 3.2.4).

The abiotic formation of noticeable amounts of methylated and non-methylated arsenic hydrides in the presence of $CH_3Cob(III)$ or aquocobalamin, respectively, was demonstrated previously (Schrauzer et al. 1973). DTE and Zn/NH_4Cl added to the *in vitro* assays were suggested as the operative electron donors in the observed hydride generation reactions with Cob(I) acting as a mediator. However, in this thesis the formation of arsenic, selenium and antimony hydrides requires no addition of an additional reductant to the *in vitro* assays. The accumulated Cob(I) formed as an end product of the MtaA catalyzed HS-CoM methylation is a conceivable reductant with $E'_{0\ Cob(I)/Cob(II)}$: -610 mV, (Kreft and Schink 1994). The high potential of Cob(I) as an reductant is reflected by its capability to catalyze numerous dehalogenation reactions (Banerjee and Ragsdale 2003). In the case of arsenite hydride generation observed in the *in vitro* assays performed here, the formation of Cob(II) from Cob(I) upon addition of high arsenite amounts (see Fig. 3.16) points towards the involvement of Cob(I) as an electron donor, presumably in order to reduce a hydronium ion to facilitate the hydride generation. That Cob(I) alone is capable to form arsine from arsenite has been demonstrated recently by adding arsenite to electrochemically formed Cob(I) under anaerobic conditions (Oliver Würfel, Ph. D. thesis, in preparation). The headspace analyses of reaction vessel by PT-GC-ICP-MS analytic revealed the formation of arsine as the sole volatile arsenic derivative.

4.1.3.4. *Proposed pathway of selenium and tellurium methylation and hydride generation*

The methylation and hydride generation pathway of selenium as representative of Group 16 elements selenium and tellurium can not be concluded with certainty from

the data of this study. The change of oxidation states of selenium in solution could not be analyzed by HG-PT-GC-ICP-MS as selenate is deprotonated in aqueous solutions (pK_a: <0) (Howard 1997). Hence, selenate is not attacked by sodium tetrahydroborate without previous reduction to selenite. However, a few aspects point towards a similar methylation and hydride generation mechanism for Group 15 and 16 metal(loid)s.

First, selenite and tellurite but neither selenate nor tellurate are converted into volatile methyl and hydride derivatives by the analyzed *in vitro* assays, indicating that the formed Cob(I) is not capable to reduce hexavalent Group 16 species. Secondly, no direct transfer of a methyl group from CH_3Cob(III) to the tetravalent selenite in the absence of HS-CoM is observed under physiological pH, neither abiotically nor in the presence of MtaA. The UV-Vis analyses of CH_3Cob(III) revealed no change of the CH_3Cob(III) typical absorption spectrum upon addition of a 2.5 excess of selenite relative to the applied CH_3Cob(III) (Fig. 3.15). HS-CoM is presumably required to enable Cob(I) formation. Cob(I) than mediates methylation and hydride generation of selenite and tellurite similarly as in the case of Group 15 elements (see section 4.1.3.1).

However, the redox state of formed volatile methyl and hydride derivatives of selenium and tellurium have redox numbers lower than of the tetravalent derivatives in solution. Thus, reduction reactions must play a role for the volatilization of these metalloids. The formed methylselenol (CH_3SeH) which is detected in notable quantities in the headspace of the performed *in vitro* assays can spontaneously react to form dimethyldiselenide ($(CH_3)_2Se_2$) as has been demonstrated previously (Gabel-Jensen et al. 2010).

4.1.4. Analyses of transcriptional response to bismuth and arsenic revealed no alternative metal(loid) methylation pathway

The *in vitro* assays performed with purified recombinant methyltransferase MtaA revealed high capability of central methyl transfer reactions from methylotrophic methanogenesis in metal(loid) volatilization via methylation and hydride generation. To decide whether alternative metal(loid) methylation pathways are involved in metal(loid) methylation *in vivo* or the CH_3Cob(III) dependent transmethylation reaction from methylotrophic methanogenesis is the primary source of metal(loid) methylation, transcriptome analyses of *M. mazei* exemplarily exposed to $Bi(NO_3)_3$

and KH_2AsO_4 were performed. The transcriptome analyses covered whole genome microarray analyses as well as northern blot analyses. In the case of microarray analyses of *M. mazei* exposed to KH_2AsO_4 it must be noted that the data were derived from a single experiment - including one dye swap reaction using the same total RNA - and must therefore be regarded as preliminary. The northern blot analyses were performed to validate the microarray data in the first place. Additionally, northern blot analyses were used to study whether a designated *arsM orf* (MM_2243) is regulated in response to arsenic.

4.1.4.1. *Metal(loid)s do not affect expression of methylcobalamin utilizing methyltransferases*

The biochemical and mechanistic studies performed in this thesis suggest a close link between multi-metal(loid) methylation and the energy metabolism of *M. mazei*. This study demonstrate the involvement of the two vitamin B_{12} derivatives $CH_3Cob(III)$ and Cob(I) in the metal(loid) methylation. MtaA is a decisive enzyme in the studied *in vitro* metal(loid) methylation and hydride generation reactions as it facilitates the formation of Cob(I) by demethylating $CH_3Cob(III)$ in a HS-CoM dependent reaction. There are numerous *orf*s in *M. mazei* potentially encoding enzymes involved in the different methanogenic pathways which catalyze the demethylation of $CH_3Cob(III)$ and thereby forming Cob(I) (refer to section 1.1.4.4). Previous transcriptional analyses suggest expression of these enzymes in response to the available methanogenic substrates (Ferguson et al. 1996; Harms and Thauer 1996; Paul and Krzycki 1996; Hovey et al. 2005; Kratzer et al. 2009). Here, it was tested whether *orf*s encoding these $CH_3Cob(III)$ dependent methyltransferases from methanogenesis show also elevated expression in response to high bismuth and arsenic concentrations and whether alternative metal(loid) methylation pathways exist.

The expression of $CH_3Cob(III)$ dependent methyltransferases is apparently not influenced by bismuth or arsenic (Tab. 3.5 and Tab. 3.7). The performed northern blot analyses could nevertheless confirm the constitutive expression of MtaA (MM_1070) in methanol grown cells, exposed or not exposed to bismuth. Only one *orf* presumably encoding one out of eight subunits of the membrane associated CH_3-H_4SPT:CoM methyltransferase showed elevated expression in response to 100 µM arsenate. The affected subunit, subunit A (MM_2251), is homologous to the corrinoid

binding subunit of the enzyme complex (Deppenmeier 2002; Deppenmeier et al. 2002) but not to the subunit H of H_4SPT:CoM methyltransferase with HS-CoM methylation activity. Thus, elevated expression of this *orf* does not (directly) contribute to metal(oid) methylation and hydride generation.

Additionally, ten *orf*s encoding proteins without annotated functions are up-regulated in the presence of 10 µM $Bi(NO_3)_3$. It can not be excluded that these *orf*s encode enzymes that potentially act as methyltransferases though not showing sequence homology to known methyltransferases.

4.1.4.2. *Triggered cob(I)alamin remethylation in response to bismuth*

In order to facilitate the next to last methyl transfer reaction in the methylotrophic branch of methanogenesis prior to methane release - the methylation of HS-CoM - CH_3Cob(III) must be provided (Harms and Thauer 1996). The *in vitro* assays with cell crude extracts from *M. mazei* as well as with purified recombinant MtaA suggest that CH_3Cob(III) is also required for metal(loid) methylation. Upon the demethylation of CH_3Cob(III), Cob(I) is formed which must be remethylated before the next HS-CoM methylation or metal(loid) methylation step can take place. The enzymes catalyzing the remethylation of Cob(I) to CH_3Cob(III) are substrate specific (Deppenmeier 2002). MtaB catalyzes the remethylation of Cob(I) from methanol, MttB from trimethylamine, MtbB from dimethylamine, MtmB from monomethylamine and MtsA from dimethylsulfide. The *orf*s encoding these methyltransferases form a transcriptional unit with genes encoding corrinoid binding proteins as indicated by several transcriptome studies of *M. mazei*, *M. barkeri* and *M. acetivorans* (Paul and Krzycki 1996; Sauer et al. 1997; Burke et al. 1998; Paul et al. 2000; Hovey et al. 2005; Bose et al. 2006; Kratzer et al. 2009). For some of these enzymes, two to three copies of the encoding genes were identified on the genome of *M. mazei* (Deppenmeier et al. 2002).

The transcriptome analyses revealed induction effects by bismuth for *orf*s encoding some of these enzymes and their adjacent *orf*s encoding the corrinoid binding proteins (Tab. 3.4). Elevated concentrations of bismuth apparently provoke elevated remethylation of Cob(I) by methanol and methylamine specific methyltransferases, forming CH_3Cob(III) by methylating Cob(I).

The *in vitro* assays performed in this thesis to demonstrate metal(loid) methylation were performed with free CH_3Cob(III). However, *in vivo* CH_3Cob(III) is presumably

mostly protein-bound. So far, it can not be decided whether bonding of $CH_3Cob(III)$ (and the corresponding Cob(I)) to specific enzymes influences metal(loid) methylation and hydride generation. To answer this question, a heterologously expressed methanol dependent methyltransferase MtaB and its associated corrinoid-binding protein MtaC from *M. mazei* is currently purified. Whether MtaB and MtaC influences the here observed multi-metal(loid) methylation and hydride generation will be tested then.

However, previous studies showed that MtaB and methanol reduces the K_m value of MtaA notably, indicating elevated HS-CoM specificity of MtaA in the presence of MtaB and methanol (Sauer and Thauer 1999). The increased specificity of the HS-CoM methylation reaction catalyzed by MtaA may contribute to the energy metabolism by minimizing the loss of methyl groups due to bismuth methylation. *M. mazei* forms high quantities of non-volatile methyl bismuth compounds in addition of volatile $(CH_3)_3Bi$ (see section 3.4 and 4.2.1). From this bismuth methylation process, the cell may suffer a mentionable loss of methyl groups. This loss is likely compensated by accelerated consumption of methanogenic substrates, enabled by the increased expression of methanol, trimethyl- and dimethylamine specific Cob(I) remethylating methyltransferases including their accompanied corrinoid binding enzymes. The methylated amines may be formed by degradation of cell compounds of cells not withstanding elevated bismuth concentrations, delivering thereby additional methanogenic substrate.

The observed formation of arsenic, selenium and antimony hydrides by *in vitro* assays containing cell-free crude extracts of *M. mazei* or recombinant MtaA requires presumably higher quantities of Cob(I) relative to $CH_3Cob(III)$ to favor hydride generation over metal(loid) methylation. The accelerated remethylation of Cob(I) induced by the presence of bismuth could thus explain why growing *M. mazei* cultures amended with bismuth formed no hydride derivatives of As, Se and Sb (Meyer et al. 2008). Nevertheless, observed formation of metal(loid) hydrides by other methanoarchaea like e.g. *Mb. formicicum* could be triggered by high quantities of other reducing cofactors like reduced F_{420} or reduced ferredoxin, presumably available in higher quantities in these methanoarchaea than in *M. mazei* grown on methanol.

4.1.4.3. *Elevated HS-CoM formation and heterodisulfide cleavage in response to bismuth*

HS-CoM plays a central role as the terminal methyl carrier prior formation of methane in all methanogenic pathways (Deppenmeier 2002). The presence of HS-CoM is also indispensable for the formation of volatile methyl and hydride metal(loid) derivatives as revealed by the MtaA containing *in vitro* assays. The role of HS-CoM is apparently to accept the methyl groups of CH_3Cob(III) to form the decisive agent for multi-metal(loid) methylation and hydride generation, Cob(I).

Microarray analyses of *M. mazei* revealed elevated expression of *orf*s MM_0133 and MM_0134 in response to 10 µM $Bi(NO_3)_3$. Homologues of these *orf*s are supposed to be involved in HS-CoM synthesis as suggested by studies of the near *M. mazei* relative *M. acetivorans* (Graupner et al. 2000; Graham et al. 2009). Also the expression of subunits of the putative heterodisulfide reductase HdrABC (MM_0387-9) is induced by bismuth. HdrABC is involved in the regeneration of HS-CoM by reductively cleaving the CoM-S-S-CoB heterodimer.

It is possible that an elevated expression of enzymes involved in the HS-CoM *de novo* synthesis is a direct response to an elevated demand of HS-CoM. High amounts of HS-CoM may accelerate bismuth methylation according to the multi-metal(loid) methylation and hydride generation pathway analyzed *in vitro* in this thesis or compensates depletion of HS-CoM caused by binding of bismuth to HS-CoM via a electrophilic attack of bismuth on the thiol group of HS-CoM, a preferred target of bismuth (Sadler et al. 1999). High bismuth concentration could also cause damage of numerous proteins in the cell. The cell has to expend additional energy for repair mechanisms and *de novo* synthesis of denaturized proteins to compensate this damage. Increased HS-CoM concentration can thereby promote methanogenesis under sufficiency of methanogenic substrates to increase the energy level of the cell. The elevated expression of the heterodisulfide reductase HdrABC can be interpreted in the same way.

Like in the case of increased expression of Cob(I) remethylating and Cob(I) binding enzymes (see section 4.1.4.2), it will be necessary to test multi-metal(loid) methylation and hydride generation capability of the complete MtaABC complex under variation of HS-CoM concentration to decide whether elevated HS-CoM concentration supports or hinders metal(loid) methylation and hydride generation.

4.1.4.4. *Transcriptional response of* arsR/arsM *operon of M. mazei in response to arsenite*

The genome of *M. mazei* carries two *orf*s designated MM_0611 and MM_2243 with high sequence homology to *arsM* (Deppenmeier et al. 2002; Qin et al. 2006). The gene *arsM* encodes a methyltransferase catalyzing the methylation of arsenite in an S-adenosyl methionine dependent reaction. The ArsM encoding gene is usually accompanied by an *orf* encoding an ArsR regulator. The microarray experiment with *M. mazei* revealed that neither *orf* MM_0611 nor *orf* MM_2243 (*arsM*) is significantly expressed in response to 10 µM $Bi(NO_3)_3$ or 100 µM KH_2AsO_4. Upon more detailed analyses of the expression of *orf* MM_2243 and its adjacent *orf* MM_2242 (*arsR*) by northern blot analyses, a notably higher expression of both *orf*s relative to control cultures not exposed to arsenic was only derived from cells exposed to 100 µM arsenite (4.2-fold higher expression relative to control cultures) (Fig. 3.22). The data also indicate an elevated expression of *orf* MM_2244, potentially encoding an arsenate reductase, ArsC. In contrast, no induced higher expression relative to control cultures of these *orf*s were detected when *M. mazei* was exposed to 10 µM arsenite or 100 µM arsenate.

The notable expression of ArsM at 100 µM arsenite but not at 100 µM arsenate is in accordance to previous findings showing elevated expression of the *arsR/arsM* operon of *R. palustris* at 0.1 mM arsenite and at 1 mM arsenate (Qin et al. 2006). It can be concluded that ArsM encoded by *orf* MM_2243 is apparently not responsible for the methylation of arsenic by *M. mazei* observed at concentrations of 1 µM KH_2AsO_4 (Meyer et al. 2008). It is more likely that at low arsenic concentrations the metal(loid) methylation proceeds via the CH_3Cob(III)/Cob(I) system, originating from methanogenesis.

4.1.5. The observed metal(loid) methylation and hydride generation pathway is presumably a common principal among all methanoarchaea

The discovered multi-metal(loid) methylation and hydride generation mechanism requires no expression of neither metal(loid) specific methyltransferases nor of metal(loid) specific reductases in order to facilitate the methylation and hydride generation of the analyzed metal(loid)s. Instead, three cofactors are required for the non-oxidative metal(loid) methylation and hydride generation pathway. These three cofactors, Cob(I), CH_3Cob(III) and HS-CoM, can be considered as standard

accessories in all methanoarchaea. Hence, the discovered multi-metal(loid) methylation and hydride generation pathway may represent a common principal in all methanoarchaea. HS-CoM is thereby required to form Cob(I). Cob(I) delivers electrons to enable metal(loid) methylation and hydride generation. Cob(I) is thereupon oxidized to Cob(II). As Cob(I) in general oxidizes to Cob(II) by auto-oxidation processes regardless of the environmental conditions, it must be perpetually reduced by the enzyme RamA (Ferguson et al. 2009). Thus, no elevated expression of additional Cob(II) reducing enzymes needs to be expected.
However, other reduced cofactors which are operative in different methanogenic pathways might also be operative in metal(loid) methylation and hydride generation and might influence the pattern of volatile metal(loid) derivatives formed like e.g. the notable formation of metal(loid) hydrides *in vivo* by *Mb. formicium* but not by *M. mazei* when grown on methanol (Meyer et al. 2008). Future research on the versatility of methanoarchaea to form methyl and hydride derivatives of metal(loid)s should therefore focus on the influence of other reduced cofactors typical for different methanogenic pathways.

4.2. Exposure of *M. mazei* to bismuth or arsenic mainly affects the energy and iron metabolism

4.2.1. Formation of non-volatile methylated bismuth derivatives is a considerable sink for methyl groups

The formation of about 120 pmol volatile $(CH_3)_3Bi$ within 48 h by 50 ml *M. mazei* pure cultures incubated with 10 µM $Bi(NO_3)_3$ has been reported previously (Thomas 2006). However, no formation of volatile $(CH_3)_3Bi$ was detected in the presence of 100 µM $Bi(NO_3)_3$. As discussed in this thesis, the observed multi-metal(loid) methylation by *M. mazei* is most likely linked to methanogenesis. The methane formation was found to be about eight orders of magnitude higher than the formation of volatile permethylated $(CH_3)_3Bi$ (Thomas 2006), pointing towards a relative low cost for the cell to methylate bismuth.
In this thesis, it was suggested that at least 30 nmol or 80 nmol of non-volatile methylated bismuth derivatives are formed within 48 h by 50 ml *M. mazei* cultures exposed to 10 or 100 µM $Bi(NO_3)_3$, respectively. From the high amounts of non-

volatile methylated bismuth derivatives relative to the volatile $(CH_3)_3Bi$ formed by *M. mazei* it must be concluded that the loss of methyl groups due to bismuth methylation is higher as previously assumed. High bismuth concentrations presumably lower the energy yield derived from methanogenesis by intercepting methyl groups actually intended to be used for a key reaction of methanogenesis, the methylation of HS-CoM. When the cell is not capable to compensate this effect of high bismuth concentration for example by accelerating the consumption of methanogenic substrates as indicated by increased expression of methylotrophic substrate specific methyltransferases and the HS-CoM synthesizing/CoM-S-S-CoB cleaving enzymes (refer to section 4.1.4.2 and 4.1.4.3), the cell growth is notably retarded as shown for *M. mazei* exposed to 100 µM $Bi(NO_3)_3$ Fig. 3.17.

4.2.2. Elevated proteolytic activity induced by bismuth and arsenic

The exposure of *M. mazei* to 10 µM $Bi(NO_3)_3$ induce expression of *orf* (MM_0483) encoding a small heat shock protein with sequence homology to a chaperon and of *orf* MM_1256 potentially encoding a CdcH protein (Deppenmeier et al. 2002). The CdcH protein shows sequence homologies to proteasome-activating nucleotidases (Deppenmeier et al. 2002). Both *orf*s showed also elevated expression in response to elevated arsenate concentration. As the products of both *orf*s are potentially involved in the degradation or refolding of misfolded enzymes, elevated bismuth and arsenic concentration may provoke increased protein damage.

4.2.3. Decrease of glutamine synthetase expression hints to elevated energy demand caused by bismuth

Elevated bismuth concentrations apparently causes loss of methyl groups (refer to section 4.2.1) actually intended for use in methanogenesis. Furthermore, high bismuth concentration causes elevated energy demand to compensate bismuth caused protein damage (see section 4.2.2). An accelerated consumption of methanogenic substrates as indicated by the elevated Cob(I) remethylation (see section 4.1.4.2) and cleavage of the CoM-S-S-CoB heterodisulfide (see section 4.1.4.3) may compensate the elevated energy demand as long as the substrate is not limiting. Nevertheless, reduction of dispensable energy consuming physiological

pathways is beneficial in natural habitats where growth substrates are usually restricted.

The transcriptome analyses of *M. mazei* exposed to 10 µM $Bi(NO_3)_3$ indicated down-regulation of *orf*s encoding glutamine synthetases $GlnA_1$ and $GlnA_2$ (MM_0964 and MM_3188). The expression of $GlnA_1$ (MM_0964) under nitrogen limitation of *M. mazei* supports the assumption that MM_0964 encodes a functional GlnA (Veit et al. 2006). In contrast, no regulation of *orf* MM_3188 ($GlnA_2$) in response to nitrogen availability was detected in *M. mazei*. Hence, the function of $GlnA_2$ is not assured. However, the GlnA catalyzed formation of glutamine from glutamate and ammonium is an ATP consuming process (Cohen-Kupiec et al. 1999). As nitrogen is not limiting under the experimental conditions, the ATP consuming ammonium fixation is presumably repressed to save energy.

4.2.4. Bismuth and arsenic trigger the expression of putative iron transporter in *M. mazei*

Four *orf*s, MM_1041, MM_1516, MM_2576 and MM_2577, potentially encoding transport proteins, show elevated expression in the presence of bismuth and arsenic. The latter both *orf*s show homology to genes encoding ferrous iron transport proteins (Deppenmeier et al. 2002). Bismuth was shown to bind to human ferric iron transporter transferrin (Sadler et al. 1999). Also the binding of bismuth to prokaryotic ferric binding proteins was assumed. The genome of *M. mazei* carries some *orf*s with the potential to encode such ferric iron transporter like the ABC iron transporter related to the enterobactin transport system of *E. coli* (MM_2067-9) (Deppenmeier et al. 2002; Grass 2006). The elevated bismuth concentration could interact with one of such ferric iron transporter in *M. mazei*. As a result, the cell could suffer from iron insufficiency. To overcome the depletion of iron in the cell, the elevated expression of the ferrous iron transporter – presumably less affected by bismuth – could be a mitigation strategy of the cell. The elevated uptake of ferrous iron could also be a mitigation strategy against elevated arsenate concentrations as ferrous iron efficiently coprecipitates with arsenate upon oxidation as demonstrated for anaerobic ferrous iron oxidizing bacteria (Hohmann et al. 2010). On the other hand, arsenic and bismuth could negatively affect iron dependent metalloproteins. Thus, an elevated uptake of iron in order to compensate the arsenate caused damage would be necessary.

5.Summary

Formation of methyl and hydride metal(loid) compounds of the elements arsenic, selenium, antimony, tellurium and bismuth is widespread in the environment – especially under anaerobic conditions – and lead to a notable modulation of mobility and toxicity of these metal(loid)s (Bentley and Chasteen 2002; Chasteen and Bentley 2003). The capability to form volatile methyl metal(loid)s – and in some cases also volatile hydrides – was shown for numerous methanoarchaea (Meyer et al. 2008). This thesis aimed towards elucidating the cause of the exceptional versatility of methanoarchaea to form metal(loid) methyl and hydride derivatives.

The comparison of inorganic metal(loid) reactants converted into volatile derivatives by cell-free crude extracts prepared from non-induced *Methanosarcina mazei* cultures and by growing cultures of the same strain indicated no inductively elevated expression of enzymes to enable the observed metal(loid) methylation reaction. The *in vitro* assays needed only amendment by two central cofactors of methanogenesis, methylcobalamin (CH_3Cob(III)) and 2-mercaptoethanesulfonate (HS-CoM), to volatilize the same metal(loid)s by methylation as growing cultures upon metal(loid) addition. Surprisingly, not only formation of volatile permethylated species like in the case of *M. mazei in vivo* but also formation of volatile hydride derivatives of arsenic, selenium and antimony was found.

As both, CH_3Cob(III) and HS-CoM are required for the observed multi metal(loid) methylation and hydride generation by cell-free crude extract but no multi-metal(loid) methylation and hydride generation by these cofactors alone were observed, the additional requirement of an enzyme using both cofactors was indicated. The CH_3Cob(III)/HS-CoM dependent methyltransferase MtaA as an integral part of the methylotrophic methanogenesis from methanol was thereupon tested whether it is capable to substitute for the cell-free crude extract. Substitution of cell-free crude extracts by MtaA results in the formation of the same volatile metal(loid) derivatives as formed in the presence of the cell-free crude extracts. This finding supports the assumed connection between metal(loid) methylation and methanogenesis and additionally demonstrates that multi metal(loid) methylation and hydride generation can arise from a key reaction of methanogenesis, the CH_3Cob(III) dependent HS-CoM methylation. The discovered multi-metal(loid) methylation and hydride generation mechanism from *M. mazei* might thus represent a common principal for

metal(loid) methylation and in some cases also for hydride generation in all methanoarchaea.

The closer analyses of arsenic methylation and hydride generation by MtaA in the presence of CH_3Cob(III) and HS-CoM revealed a decisive role of the CH_3Cob(III) demethylation product Cob(I). Moreover, the data from the performed experiments points towards a non-oxidative methylation mechanism. The role of Cob(I) is probably to supply electrons to enable the methylation and hydride generation reactions.

Analyses of the transcriptional response of *M. mazei* towards bismuth and arsenic were performed to investigate whether the discovered *in vitro* multi-metal(loid) methylation pathway is also feasible for the observed *in vivo* multi metal(loid) methylation. The analyses revealed no expression of genes that hint to an alternative pathway leading to multi-metal(loid) methylation. Even the *arsR/arsM* operon of *M. mazei* which was previously described as being capable to form volatile methyl arsenic derivatives from arsenite (Qin et al. 2006; Yuan et al. 2008) is not inductively more expressed at arsenite and arsenate concentrations at which notable methylation by *M. mazei* was demonstrated previously (Meyer et al. 2008). Instead, genes encoding enzymes participating in cob(I)alamin remethylation and HS-CoM recycling/de-novo syntheses were noticeably up-regulated in the presence of bismuth, thus supporting the assumption that multi-metal(loid) methylation also *in vivo* is directly coupled to methanogenesis. The transcriptional analyses also indicate some general stress responses towards exposure to elevated bismuth and arsenic concentrations and points towards imbalance of the iron metabolism in *M. mazei*.

Open to question is whether Cob(I) is the only electron donor *in vivo* that enables the multi-metal(loid) methylation and hydride generation or whether other reduced cofactor are capable to trigger the observed *in vivo* multi metal(loid) methylation as well.

6. Zusammenfassung

Die Bildung von Methyl- und Hydridderivaten der Metalle und Metalloide (Metall(oid)e) Arsen, Selen, Antimon, Tellur und Bismut ist ein weit verbreitetes Phänomen in der Umwelt, welches sowohl die Mobilität als auch die Toxizität der jeweiligen Metall(oid)e deutlich beeinflusst (Bentley and Chasteen 2002; Chasteen and Bentley 2003). Die Fähigkeit zur Bildung von Methyl- und Hydridderivaten der oben angeführten Metall(oid)e konnte bereits für eine Reihe von Mikroorganismen aus der Gruppe der Methanoarchaea nachgewiesen werden (Meyer et al. 2008). Die vorliegende Arbeit hat die Zielsetzung den Grund für diese außergewöhnliche Fähigkeit der Metall(oid) Methylierung und Hydrierung aufzudecken.

In dieser Arbeit wird am Beispiel von *Methanosarcina mazei* gezeigt, dass keine Induktion von spezifischen Enzymen, ausgelöst durch Inkubation mit hohen Metall(loid) Konzentrationen, erforderlich ist, um die Bildung flüchtiger Methyl und Hydrid Metall(oid) Derivate zu ermöglichen. Den durchgeführten *in vitro* Versuchen mit zellfreiem Rohextrakt von *M. mazei* kann entnommen werden, dass neben dem zellfreien Rohextrakt lediglich die Anwesenheit zweier zentraler Kofaktoren der Methanogenese, nämlich 2-Mercaptoethansulfonate (HS-CoM) und Methylcobalamin (CH_3Cob(III)), notwendig ist. Weiterführende Experimente mit einer aufgereinigten, rekombinanten Methyltransferase MtaA, die eine zentrale Rolle in der methylotrophen Methanogenese spielt (Grahame 1989; Ferguson et al. 1996; Harms and Thauer 1996) zeigen, dass das stark reduzierende Demethylierungsprodukt von CH_3Cob(III), Cob(I)alamin, eine wichtige Rolle sowohl in der Metall(oid) Methylierungs- als auch Hydrierungsreaktion spielt. Die ermittelten Daten deuten auf den Transfer von Methylcarbanion von CH_3Cob(III) auf das jeweilige Metal(loid) hin. Cob(I) liefert hierbei höchstwahrscheinlich die erforderlichen Elektronen um diesen Methylgruppen Transfer zu ermöglichen.

Das die aus den *in vitro* Experimente gewonnen Erkenntnis, dass die Multi-Metall(oid) Methylierung und Hydrierung in *M. mazei* direkt mit dem zentralen Energiestoffwechsel der Zelle, der Methanogenese, *in vivo* gekoppelt ist, kann mit Hilfe von globalen Genexpressionsstudien untermauert werden.

Aufgrund der in dieser Arbeit gewonnen Daten wird vorgeschlagen, dass der hier beschrieben Weg zur Bildung von flüchtigen Metall(oid) Derivaten ein gemeinsames Prinzip aller Methanoarchaea darstellt.

7. References

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8. Abbreviations

A. bidest.	aqua bidestillata = two times distilled water
Amp^r	ampicillin resistence
approx.	approximately
APS	ammonium persulfate
bp	base pair(s)
BSA	bovine serum albumin
c	centi (10^1)
$CH_3Cob(III)$	methylcob(III)alamin
CH_3-S-CoM	methyl-2-mercaptoethanesulfonate
CIAP	calf intestinal alkaline phosphatase
CoA	coenzyme A
Cob(I)	cob(I)alamin
Cob(II)	cob(II)alamin
cps	counts per second
Cy3-dCTP	5-amino-propargyl-2'-deoxy-cytosine-5'-triphosphate with Cy3-fluorochrome (fluorescence at 535 nm)
Cy5-dCTP	5-amino-propargyl-2'-deoxy- cytosine-5'-triphosphate with Cy5-fluorochrome (fluorescence 635 nm)
DEPC	diethylpyrocarbonate
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
dsDNA	double-stranded DNA
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen = German Collection of Microorganisms and Cell Cultures
DTT	dithiothreitol
DTE	dithioerythrol
e.g.	Exempli Gratia (= for example)
EDTA	ethylene-diamine-tetraacetic acid
EI-MS	electron impact-mass spectrometer
endconc.	endconcentration
et al.	et alteri = and others
etc.	*et cetera*

Fig.	figure
FPLC	fast protein liquid chromatography
g	gram
x g	gravitational acceleration
GSH	glutathione
h	hour
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HG	hydride generation
His_6-tag	hexa histidine tag
H_4MPT	tetrahydromethanopterin
hPa	hectopascal
HS-CoB	N-7-mercaptoheptanoyl-L-threonine phosphate
HS-CoM	2-mercaptoethanesulfonate
H_4SPT	tetrahydromethansarcinapterin
ICP-MS	inductively coupled plasma-mass spectrometer
i.d.	inner diameter
i.e.	id est = that is, that is to say
IPTG	isopropyl-ß-D-thiogalactopyranoside
IUPAC	International Union of Pure and Applied Chemistry
Kan^r	kanamycin resistence
kb	kilobases
kDa	kilodalton
L	liter
LB	Luria-Bertani
M	molar (mol L^{-1})
m	milli (10^{-3})
m	meter
m/z	mass per charge ratio
µ	micro (10^{-6})
µCi	micro curie
mA	milliampere
MFR	methanofuran
min	minute
MOPS	3-(N-morpholino)propanesulphonic acid

n	nano (10^{-9})
NADP+	nicotinamide adenine dinucleotide phosphate
NCBI	National Center for Biotechnology Information
Ni-NTA	nickel-nitrilotriacetic acid
NMR	nuclear magnetic resonance
NR	not reported
OD	optical density
orf	open reading frame
p	pico (10^{-12})
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
pH	negative decimal logarithm of the hydronium ion (H_3O^+) concentration
pK_a	negative decimal logarithm of the acid dissociation constant
ppb	parts per billion
ppm	parts per million
psi	pound-force per square inch
PT-GC	purge&trap gas chromatography
Pfu-Polymerase	DNA-polymerase from Pyrococcus furiosus
R	moiety
RNA	ribonucleic acid
RNase	ribonuclease
rRNA	ribosomal RNA
rNTP	ribonucleoside triphosphate
rpm	rounds per minute
RT	room temperature
s	seconds
SAM	S-adenosyl methionine
SDS	sodiumdodecylsulfate
SRB	sulfate reducing bacteria
SSC	standard saline citrate
ssDNA	single-stranded DNA
Tab.	table

TAE	tris-acetate-EDTA buffer
Taq-Polymerase	DNA-Polymerase from Thermus aquaticus
TEMED	N,N,N',N'-Tetramethylethylenediamine
$T_{annealing}$	annealing temperature
Tm	melting temperature
Tris	tris-(hydroxymethyl)-aminomethane
U	(enzyme) unit = 1 µmol substrate converted in 1 min (unless otherwise indicated).
UV	ultraviolet
UV-Vis	ultraviolet-visible spectroscopy/ spectroscopic
V	volt
Vol	volume
W	watt
www	world wide web
x	fold
λ	wavelength
<	lower than
>	higher than
%	per cent (10^{-2})
% (v/v)	percent by volume
% (w/v)	percent by weight

9. Acknowledgements

The compilation of a Dissertation – especially when working experimentally – is nearly impossible without the dedicated help of some fellow men. Hence, I like to express my gratefulness to some people who made this work possible:

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10. Appendix

Tab. 10.1: Boiling points of analytes detected, not listed in Tab. 2.11.

Compound	Boiling Point [°C]	Reference
$(CH_3)_3Bi$	108	(Feldmann and Hirner 1995)
$(CH_3)_2Te$	82	(Feldmann and Hirner 1995)
$(CH_3)_2Te_2$	196	(Swearingen et al. 2004)
$(CH_3)_2SbH$	61	(Andrewes and Cullen 2003)
$(CH_3)_3Sb$	82	(Feldmann and Hirner 1995)
CH_3SeH	25.5	(Briggs et al. 1953)
$(CH_3)_2AsH$	38	(Feldmann and Hirner 1995)
$(CH_3)_3As$	57	(Feldmann and Hirner 1995)

Tab. 10.2: Formation of volatile and non-volatile methylated arsenic compounds from trivalent and pentavalent arsenic reactants by MtaA containing *in vitro* assays.
The formation of volatile and non-volatile methylated As derivatives by *in vitro* assays containing 10 µg MtaA (MM_1070), 1 µmol $CH_3Cob(III)$ and HS-CoM and 1 mL 50 mM HEPES pH 7 upon addition of tri- and pentavalent arsenic reactants was investigated. Volatile species were analyzed by headspace PT-GC-ICP-MS and non-volatile species by HG-PT-GC-ICP-MS. Chemical hydride generation using sodium borhydride was separately performed at neutral pH (pH 7 – 8) and during pH gradient by continuous addition of 1 M HCl in order to differentiate between trivalent and pentavalent arsenic derivatives. Reduced and methylated products are in boldface, reactants not transformed in the in the MtaA containing *in vitro* assays are in italics. Blank values derived from chemical hydride generation without addition of *in vitro* assays were subtracted. Experiments were performed at least in triplicates. For calculation of total volatilization, all volatile species detected for each experiment were summarized first. Means and relative standard deviations were calculated from these summarized values.

		$AsNaO_2$	$CH_3AsO(ONa)_2$	CH_3AsI_2	$(CH_3)_2AsO(OH)$
	reactants added [pmol] (± %SD)*	9 304 (± 2%)	57 636 (± 5%)	877 (± 2%)	1 616 (± 2%)
methylation and hydrogenation by *in vitro* assays containing MtaA					
total volatilized	[pmol] (± %SD)	1 302 (±12%)	2.6 (±20%)	307 (±12%)	3.1 (±38%)
	[%]	14	<0.01	35	0.19
total methylation‡	[pmol] (± %SD)	**1 201 (±2%)**	**2.8 (±14%)**	**159 (±10%)**	**11 (±26%)**
	[%]	**13**	**<0.01**	**18**	**0.7**
recovery	[%]	76	105	69	110
amounts of methylated and hydrogenated arsenic derivatives as detected by headspace and HG-PT-GC/ICP-MS analyses [pmol] (±%SD)					
trivalent volatile species	AsH_3	689 (±15%)	<DL	<DL	<DL
	CH_3AsH_2	**514 (±11%)**	2.0 (±18%)	205 (±14%)	<DL
	$(CH_3)_2AsH$	**68 (±11%)**	**0.52 (±27%)**	**91 (±10%)**	2.5 (±53%)
	$(CH_3)_3As$	**3.8 (±4%)**	**0.045 (±36%)**	**12.3 (±15%)**	**0.36 (±59%)**
	As_u	27 (±20%)	<DL	1.3 (±0.1%)	<DL
trivalent non-volatile species	$As(OH)_3$	*4 870 (±10%)*	73 (±56%)	6.0 (±26%)	7.7 (±47%)
	$CH_3As(OH)_2$	**521 (±13%)**	103 (±1%)	*141 (±18%)*	2.5 (±58%)
	$(CH_3)_2As(OH)$	**35 (±21%)**	**0.62 (±6%)**	**40 (±28%)**	9.4 (±10%)
pentavalent non-volatile species	$AsO(OH)_3$	288 (±12%)	194 (±6%)	49 (±16%)	64 (±37%)
	$CH_3AsO(OH)_2$	**45 (±8%)**	*60 106 (±6%)*	20 (±20%)	9.2 (±20%)
	$(CH_3)_2AsO(OH)$	**13 (±6%)**	<DL	**22 (±62%)**	*1671 (±7%)*
	$(CH_3)_3AsO$	**2.0 (±15%)**	<DL	**1.6 (±10%)**	**9.4 (±33%)**

<DL: below detection limit

*Amounts of reactants added based on the concentrations of reactants determined by HG-PT-GC/ICP-MS.

‡Only derivatives with a higher methylation grade than the added reactants were considered.

As_u: unknown volatile arsenical

Tab. 10.3: List of the microarray data derived from five individual experiments performed with *M. mazei* cultures exposed to 10 µM $Bi(NO_3)_3$ or 30 µM KNO_3.
Only data derived from at least three individual experiments, including one dye swap reaction, were considered. Data already listed in the result section are excluded.

Orf No.	Annotated geneproduct	Position on the chromosome	mean expression ratios 10 µM Bi vs. control (log2)	standard deviation of mean expression ratios (log2)
MM_0002	Dipeptide ABC transporter, binding protein	2677 _ 4281	-0.36	0.29
MM_0004	Dipeptide ABC transporter, permease protein	4615 _ 5604	-0.33	0.29
MM_0005	Dipeptide ABC transporter, permease protein	5630 _ 6484	-0.27	0.26
MM_0006	Dipeptide ABC transporter, ATP-binding protein	6523 _ 7497	-0.32	0.62
MM_0007	Dipeptide ABC transporter, ATP-binding protein	7490 _ 8098	-0.24	0.26
MM_0010	Hypothetical protein	11299 _ 11090	-0.01	0.28
MM_0011	Conserved protein	12748 _ 13152	-0.31	0.26
MM_0013	Conserved protein	15146 _ 15634	-0.24	0.14
MM_0014	Undecaprenyl pyrophosphate synthetase	16177 _ 16545	0.19	0.52
MM_0017	Ubiquinone/menaquinone biosynthesis methyltransferase	20581 _ 19763	-0.02	0.37
MM_0018	Conserved protein	21863 _ 22198	0.21	0.79
MM_0019	Conserved protein	23977 _ 22685	0.08	0.52
MM_0020	Conserved protein	25307 _ 24300	-0.23	0.11
MM_0021	Conserved protein	25929 _ 26183	-0.03	0.56
MM_0024	Hypothetical protein	27399 _ 27707	0.16	0.44
MM_0026	Conserved protein	29472 _ 28930	0.11	0.18
MM_0027	Conserved protein	30167 _ 29823	-0.32	0.27
MM_0032	Conserved protein	36605 _ 36826	0.21	0.48
MM_0034	Hypothetical protein	38780 _ 37839	-0.07	0.28
MM_0037	Argininosuccinate synthase	45847 _ 44606	0.25	0.13
MM_0038	Carbamoyl-phosphate synthase large chain	49390 _ 46172	0.10	0.22
MM_0039	Carbamoyl-phosphate synthase small chain	50529 _ 49393	-0.07	0.50
MM_0040	Glycine betaine transporter, ATP-binding protein (OtaA)	51425 _ 52774	-0.10	0.40
MM_0041	Glycine betaine transporter, permease protein (OtaB)	52774 _ 53601	-0.07	0.18
MM_0043	Conserved protein	57651 _ 55012	-0.16	0.26
MM_0045	Hypothetical protein	59399 _ 60478	0.23	0.34
MM_0046	Hypothetical protein	60558 _ 61067	-0.02	0.55
MM_0047	Acetylornithine aminotransferase	63090 _ 61666	-0.06	0.42
MM_0048	Aldehyde dehydrogenase	64571 _ 63096	-0.05	0.41
MM_0050	Glutamyl-tRNA (Gln) amidotransferase	68049 _ 66151	0.01	0.47
MM_0054	Transposase	71424 _ 71879	0.59	0.28
MM_0055	Universal stress protein	73809 _ 72310	0.22	0.29
MM_0057	Tungsten formylmethanofuran dehydrogenase, su F	77008 _ 77286	-0.05	0.30
MM_0058	Tungsten formylmethanofuran dehydrogenase, su G	77762 _ 78223	0.11	0.40
MM_0059	Tungsten formylmethanofuran dehydrogenase su B	78236 _ 79477	-0.24	0.52
MM_0061	Conserved protein	80012 _ 80293	-0.11	0.58
MM_0062	Putative methyltransferase	81223 _ 80561	-0.10	0.45
MM_0063	Cell surface glycoprotein (s-layer protein)	82753 _ 81392	-0.25	0.56
MM_0064	Cell surface glycoprotein (s-layer protein)	85348 _ 83345	0.24	0.89
MM_0067	Hypothetical protein	91257 _ 89716	-0.17	0.30
MM_0068	N-methylhydantoinase	92966 _ 91257	-0.17	0.26
MM_0070	Putative ribosomal RNA methyltransferase	94842 _ 95570	0.09	0.45
MM_0072	Thermosome beta-su	98158 _ 96458	0.11	0.62
MM_0073	Ribose 5-phosphate isomerase	99273 _ 98572	-0.21	0.05
MM_0074	Aspartyl-tRNA synthetase	100720 _ 99389	-0.26	0.28
MM_0075	Conserved protein	101277 _ 101645	-0.15	0.42
MM_0080	Hypothetical protein	107258 _ 106131	0.23	0.56
MM_0082	Phosphoserine phosphatase	109408 _ 109133	-0.18	0.53
MM_0084	Shikimate kinase	110374 _ 109847	-0.40	0.55
MM_0085	Conserved protein	110974 _ 110612	0.35	0.18
MM_0086	Hypothetical protein	111917 _ 111387	-0.22	0.46
MM_0088	Putative transport protein	114763 _ 113891	0.10	0.12
MM_0089	Hypothetical protein	115342 _ 115139	0.19	0.40
MM_0092	Conserved protein	119647 _ 118502	-0.20	0.53
MM_0093	Cobyric acid synthase CbiP	120209 _ 121705	-0.06	0.16
MM_0095	Conserved protein	123133 _ 123822	-0.14	0.56
MM_0096	Transposase	124395 _ 125660	-0.05	0.40

MM_0097	Ornithine cyclodeaminase	126740 _ 125739	0.34	0.39
MM_0098	Conserved protein	126929 _ 127636	-0.17	0.43
MM_0099	Tetratrico peptide repeat protein	128300 _ 127686	0.30	0.54
MM_0100	Conserved protein	128735 _ 128358	0.10	0.57
MM_0101	Conserved protein	129381 _ 129031	-0.10	0.35
MM_0102	Serine/threonine protein phosphatase	129795 _ 130613	0.22	0.46
MM_0104	Hypothetical protein	132454 _ 132188	-0.36	0.12
MM_0106	FxsA protein	133816 _ 134217	0.00	0.51
MM_0107	Hypothetical protein	135046 _ 134333	-0.03	0.63
MM_0108	Hypothetical protein	135449 _ 135051	-0.33	0.39
MM_0109	Cysteine desulfhydrase	135605 _ 136873	-0.02	0.28
MM_0111	Ribosomal protein S6 modification protein	137429 _ 138400	-0.20	0.32
MM_0112	Hypothetical protein	138650 _ 139021	-0.05	0.05
MM_0113	Conserved protein	139146 _ 140378	0.11	0.31
MM_0114	Conserved protein	140925 _ 142238	-0.57	0.36
MM_0118	CTP synthase	148154 _ 146553	-0.33	0.15
MM_0119	Hypothetical protein	149466 _ 148705	-0.21	0.30
MM_0120	Conserved protein	149642 _ 149478	-0.22	0.43
MM_0123	Universal stress protein	153174 _ 153632	-0.30	0.44
MM_0125	Universal stress protein	155445 _ 155888	0.34	0.39
MM_0128	ATP-dependent protease La	160753 _ 158687	0.04	0.66
MM_0129	Conserved protein	161203 _ 161964	0.10	0.48
MM_0130	Conserved protein	162211 _ 161978	-0.07	0.82
MM_0135	Ferredoxin oxidoreductase	168838 _ 167525	0.01	0.33
MM_0136	Zn-dependent hydrolase	169773 _ 169168	-0.03	0.65
MM_0138	Conserved protein	173136 _ 172684	-0.30	0.24
MM_0141	Conserved protein	176529 _ 177053	-0.05	0.36
MM_0142	Orotate phosphoribosyltransferase	177146 _ 177706	-0.46	0.19
MM_0143	4-Carboxymuconolactone decarboxylase	178181 _ 177873	-0.35	0.29
MM_0144	Phosphoribosylamine--glycine ligase	178642 _ 179946	-0.19	0.30
MM_0145	Ornithine carbamoyltransferase	180064 _ 181023	-0.18	0.52
MM_0146	Transcriptional regulator	182613 _ 183395	-0.05	0.17
MM_0148	Conserved protein	184446 _ 185240	-0.21	0.23
MM_0149	Hypothetical protein	185332 _ 185541	-0.21	0.41
MM_0150	putative nucleoside-diphosphate-sugar epimerase	185898 _ 186764	-0.14	0.43
MM_0151	Conserved protein	191069 _ 189186	0.25	0.56
MM_0152	DNA integration/recombination/invertion protein	193066 _ 191933	-0.25	0.42
MM_0155	Conserved protein	200122 _ 198320	0.07	0.43
MM_0156	Type III restriction enzyme	200894 _ 204388	-0.03	0.60
MM_0157	ATP-dependent DNA helicase	204707 _ 205366	0.05	0.16
MM_0158	ATP-dependent DNA helicase	205604 _ 205975	-0.52	0.24
MM_0159	Hypothetical protein	206782 _ 207453	-0.08	0.71
MM_0160	Hypothetical protein	207938 _ 207714	-0.01	0.38
MM_0162	Conserved protein	208431 _ 209813	0.37	0.58
MM_0164	ATP-dependent protease La	212816 _ 214861	0.03	0.07
MM_0165	Hypothetical protein	215330 _ 215013	0.02	0.27
MM_0166	Homospermidine synthase	215609 _ 217054	-0.05	0.06
MM_0167	Cobalt-zinc-cadmium resistance protein	217859 _ 218947	-0.19	0.38
MM_0168	Hypothetical sensory transduction histidine kinase	223908 _ 219157	0.01	0.41
MM_0169	Hypothetical sensory transduction histidine kinase	226247 _ 223962	-0.22	0.34
MM_0173	AdoCbi amidohydrolase CbiZ	230669 _ 231268	0.27	0.55
MM_0174	Methanol corrinoid protein MtaC3	238003 _ 238782	-0.09	0.73
MM_0178	Conserved protein	243509 _ 248641	0.21	0.31
MM_0179	Conserved protein	249399 _ 249800	-0.09	0.74
MM_0182	Thiamine biosynthesis protein	253347 _ 251929	-0.02	0.30
MM_0183	DNA topoisomerase III	254024 _ 256519	0.07	0.31
MM_0184	SSU ribosomal protein S3AE	257650 _ 257039	-0.15	0.39
MM_0185	Short chain dehydrogenase/reductase	258697 _ 259461	-0.03	0.63
MM_0187	Nucleotidyltransferase	260231 _ 260539	0.48	0.37
MM_0188	Putative nucleotidyltransferase	260539 _ 260883	0.46	0.16

MM_0189	Conserved protein	261219 _ 260893	0.30	0.58
MM_0191	Transposase	266008 _ 264383	0.07	0.31
MM_0194	Conserved protein	270155 _ 270448	0.36	0.24
MM_0195	Conserved protein	270598 _ 272451	-0.43	0.36
MM_0197	Conserved protein	273320 _ 274663	-0.31	0.60
MM_0200	Conserved protein	277460 _ 277795	0.40	0.14
MM_0201	Conserved protein	277773 _ 278141	0.10	0.45
MM_0203	Hypothetical protein	278848 _ 279864	-0.06	0.54
MM_0211	Cysteine proteinase	286021 _ 288192	-0.01	0.58
MM_0212	Conserved protein	288558 _ 288322	-0.34	0.58
MM_0213	Putative single-stranded-DNA-specific exonuclease	290059 _ 288725	-0.46	0.22
MM_0214	Hypothetical protein	291807 _ 290425	0.06	0.36
MM_0216	Conserved protein	294780 _ 293968	0.07	0.53
MM_0218	Conserved protein	297837 _ 295624	0.19	0.13
MM_0219	Conserved protein	298722 _ 299852	0.23	0.47
MM_0221	Fe-S oxidoreductase	302383 _ 300662	0.02	0.19
MM_0224	Molybdenum-pterin-binding-protein	305497 _ 305703	0.03	0.87
MM_0226	Conserved protein	306684 _ 307034	0.12	0.60
MM_0229	Hypothetical protein	308700 _ 308476	0.05	0.71
MM_0230	Sodium-calcium exchanger	309343 _ 310389	-0.23	0.46
MM_0232	Hypothetical protein	312030 _ 312398	0.36	0.37
MM_0233	Alanyl-tRNA synthetase	313440 _ 312631	-0.09	0.45
MM_0235	Conserved protein	318631 _ 317357	0.38	0.67
MM_0238	Hypothetical protein	320656 _ 321378	0.02	0.22
MM_0239	Conserved protein	322308 _ 321499	0.59	0.18
MM_0240	Ferredoxin	323169 _ 322786	-0.22	0.75
MM_0241	Potential ferredoxin oxidoreductase	324674 _ 323175	-0.07	0.79
MM_0242	Glycerol-3-phosphate cytidylyltransferase	325004 _ 325456	-0.27	0.56
MM_0243	Aspartate aminotransferase	326625 _ 325486	-0.06	0.16
MM_0244	Riboflavin synthase, su beta	327083 _ 326682	-0.10	0.34
MM_0245	Riboflavin synthase, su alpha	327597 _ 327136	-0.26	0.26
MM_0247	Putative inosine monophosphate dehydrogenase	329723 _ 329163	-0.27	0.47
MM_0249	BRAM protein	333359 _ 332706	-0.08	0.48
MM_0251	Transposase	334887 _ 334606	-0.17	0.48
MM_0253	Replication factor c su	339394 _ 337565	0.30	0.19
MM_0259	Dihydroxy-acid dehydratase	347013 _ 345355	-0.08	0.22
MM_0262	Conserved protein	351776 _ 350982	-0.17	0.49
MM_0263	Conserved protein	352892 _ 352473	0.32	0.73
MM_0264	Conserved protein	353765 _ 353475	0.00	0.64
MM_0268	Conserved protein	359563 _ 359321	0.48	0.50
MM_0269	Hypothetical protein	360219 _ 360743	-0.32	0.38
MM_0274	Endonuclease (putative)	366307 _ 365843	-0.37	0.38
MM_0275	Hypothetical protein	368112 _ 366313	-0.12	0.46
MM_0276	Superfamily II DNA and RNA helicase	371942 _ 368118	0.12	0.08
MM_0277	T/G-SPECIFIC DNA GLYCOSYLASE	372837 _ 372166	-0.19	0.10
MM_0278	DNA-cytosine methyltransferase	374105 _ 372837	-0.23	0.18
MM_0279	Membrane alanine aminopeptidase	374329 _ 377322	0.06	0.57
MM_0281	Hypothetical protein	378199 _ 378543	-0.16	0.64
MM_0282	Threonine synthase	378966 _ 380180	0.00	0.13
MM_0283	Leucyl-tRNA synthetase	380460 _ 383372	-0.09	0.32
MM_0284	Amidohydrolase (putative)	384653 _ 383736	-0.12	0.45
MM_0285	Conserved protein	385388 _ 384741	0.11	0.14
MM_0286	Hypothetical protein	386031 _ 386525	-0.03	0.12
MM_0287	Deoxyhypusine synthase	386949 _ 387995	-0.12	0.55
MM_0288	Transcriptional regulator, ArsR family	388062 _ 388580	-0.10	0.34
MM_0290	Hypothetical protein	389639 _ 390184	-0.01	0.28
MM_0293	Conserved protein	392722 _ 391460	0.14	0.12
MM_0295	Glycine betaine transport system, permease protein	396194 _ 395577	-0.13	0.50
MM_0297	Glycine betaine transporter, ATP-binding protein	398207 _ 397086	-0.21	0.32
MM_0298	Glycine betaine-binding protein	399152 _ 398235	-0.09	0.39

MM_0299	Glucose-1-phosphate thymidylyltransferase	399676 _ 400905	-0.23	0.64
MM_0300	Glucosamine-fructose-6-phosphate aminotransferase [isomerizing]	400931 _ 402784	0.16	0.35
MM_0301	Phosphoglucomutase/phosphomannomutase	402843 _ 404174	0.05	0.58
MM_0302	Glucose-1-phosphate thymidylyltransferase	404174 _ 405364	0.18	0.44
MM_0304	AAA family ATPase	407340 _ 408455	0.19	0.72
MM_0306	Metal-dependent hydrolases	410159 _ 409245	-0.12	0.31
MM_0308	Uroporphyrinogen-III synthase	411390 _ 412196	-0.18	0.27
MM_0309	Metallo cofactor biosynthesis protein	412310 _ 413506	-0.11	0.67
MM_0311	Conserved protein	415207 _ 416154	-0.02	0.49
MM_0312	Hypothetical protein	416242 _ 417003	-0.06	0.44
MM_0314	Valyl-tRNA synthetase	418656 _ 421262	-0.09	0.36
MM_0318	Flagella related protein FlaH	426942 _ 426229	0.00	0.59
MM_0323	Flagellin B1 precursor	429914 _ 429270	-0.38	0.33
MM_0325	Chemotaxis protein methyltransferase CheR	431480 _ 430671	-0.03	0.64
MM_0329	Protein-glutamate methylesterase CheB	436436 _ 435366	-0.36	0.22
MM_0333	Methyl-accepting chemotaxis protein	440174 _ 438180	0.43	0.15
MM_0334	Hypothetical protein	440699 _ 441064	0.13	0.44
MM_0335	3-Hydroxy-3-methylglutaryl-coenzyme A reductase	441695 _ 442948	0.13	0.31
MM_0337	Tryptophan synthase, beta chain	445286 _ 443961	0.23	1.03
MM_0338	Putative snRNP Sm-like protein	447075 _ 445720	0.06	0.30
MM_0339	Putative snRNP Sm-like protein	447635 _ 447850	-0.06	0.37
MM_0341	Amidophosphoribosyltransferase	448215 _ 449669	-0.28	0.41
MM_0342	Hypothetical protein	450162 _ 449767	-0.13	0.22
MM_0344	Probable cation efflux pump	452906 _ 451473	-0.46	0.60
MM_0345	Transcriptional regulator	453317 _ 452991	-0.29	0.29
MM_0348	Putative cation efflux pump	456394 _ 454928	0.07	0.21
MM_0350	Putative molybdopterin biosynthesis protein	457470 _ 458207	-0.03	0.33
MM_0351	Conserved protein	458507 _ 459499	-0.45	0.47
MM_0353	Conserved protein	460760 _ 460062	0.27	0.84
MM_0356	Conserved protein	462764 _ 463786	-0.09	0.43
MM_0357	Glutamate dehydrogenase	464319 _ 465440	0.61	0.26
MM_0358	Acetyl-CoA synthetase, alpha su	467021 _ 465618	0.49	0.43
MM_0359	Hypothetical protein	467584 _ 467231	0.15	0.54
MM_0360	Sulfite reductase, assimilatory-type	467707 _ 468399	-0.28	0.22
MM_0361	Transcriptional regulator, MarR family	468816 _ 468463	0.14	0.45
MM_0363	Hypothetical protein	470083 _ 470418	0.00	0.57
MM_0364	Hypothetical protein	470459 _ 470800	0.29	0.57
MM_0365	Conserved protein	471016 _ 471474	0.24	0.60
MM_0367	Hypothetical protein	472731 _ 472180	0.08	0.43
MM_0368	Hypothetical protein	472947 _ 473261	-0.04	0.46
MM_0370	Chorismate mutase / prephenate dehydratase	474376 _ 475437	-0.12	0.17
MM_0371	Conserved protein	475505 _ 476389	-0.02	0.31
MM_0372	MoxR-like ATPase	476651 _ 477622	-0.19	0.19
MM_0373	Hypothetical protein	477794 _ 478672	0.10	0.46
MM_0374	Conserved protein	478678 _ 480552	0.01	0.23
MM_0375	Conserved protein	480552 _ 483038	0.11	0.66
MM_0376	Conserved protein	483764 _ 483171	0.13	0.55
MM_0377	Integral membrane protein	484916 _ 483867	0.44	0.24
MM_0378	Sugar-phosphate nucleotidyltransferase	486448 _ 485273	0.12	0.55
MM_0381	Peptidyl-prolyl cis-trans isomerase	488279 _ 487782	0.11	0.67
MM_0382	Peptidyl-prolyl cis-trans isomerase	488830 _ 488342	-0.03	0.51
MM_0384	Conserved protein	490071 _ 492446	-0.39	0.59
MM_0385	Hypothetical protein	494241 _ 493831	-0.16	0.50
MM_0391	Hypothetical protein	504279 _ 501997	0.08	0.21
MM_0392	Hypothetical protein similar to C-terminal domain of HdrB	504926 _ 505174	-0.13	0.34
MM_0393	Conserved protein	506238 _ 505705	-0.22	0.41
MM_0394	Conserved protein	507105 _ 506269	-0.15	0.48
MM_0395	Surface layer protein (putative)	508689 _ 507433	-0.26	0.38
MM_0396	Transposase	509905 _ 508775	0.37	0.16

MM_0398	Conserved protein	511763 _ 510618	-0.27	0.53
MM_0402	Conserved protein	515348 _ 514143	-0.04	0.70
MM_0404	Hypothetical protein	516288 _ 516079	-0.11	0.64
MM_0405	Surface layer protein (putative)	517700 _ 516270	-0.08	0.51
MM_0406	Conserved protein	519059 _ 517887	-0.13	0.26
MM_0407	Hypothetical protein similar to C-terminal domain of HdrB	519541 _ 519840	0.45	0.38
MM_0408	Hypothetical protein	520378 _ 519962	0.18	0.55
MM_0409	3-Isopropylmalate dehydratase	520855 _ 522027	-0.44	0.21
MM_0411	Conserved protein	523706 _ 522696	-0.27	0.27
MM_0414	Flagella related protein FlaH	528182 _ 527391	0.12	0.12
MM_0419	Pyruvate synthase, su beta	532543 _ 531695	0.14	0.54
MM_0420	Pyruvate synthase, su alpha	534269 _ 532533	0.11	0.49
MM_0423	Conserved protein	537590 _ 537165	-0.09	0.31
MM_0424	Histidinol dehydrogenase	538978 _ 537680	-0.16	0.05
MM_0425	Helicase (DEAD/DEAH family)	541784 _ 539595	-0.05	0.24
MM_0426	RNase L inhibitor	543724 _ 541961	-0.06	0.33
MM_0427	Hypothetical protein	544506 _ 544072	-0.11	0.35
MM_0428	Conserved protein	545658 _ 544642	-0.06	0.31
MM_0429	Type I restriction-modification system methylation su	546056 _ 547783	-0.12	0.44
MM_0430	Type I restriction-modification system specificity su	547779 _ 549095	-0.29	0.33
MM_0431	Type I restriction-modification system restriction su	549109 _ 552264	0.19	0.54
MM_0434	Conserved protein	553883 _ 554758	0.20	0.61
MM_0436	Thioredoxin	556320 _ 556057	-0.02	0.72
MM_0437	Hypothetical protein	557400 _ 556591	0.04	0.16
MM_0438	Methylenetetrahydrofolate reductase	558615 _ 557740	0.26	0.11
MM_0439	Zinc finger protein	559421 _ 558810	0.24	0.32
MM_0441	Methylenetetrahydrofolate dehydrogenase (NADP+)	562245 _ 561385	-0.46	0.54
MM_0442	Serine hydroxymethyltransferase	563529 _ 562273	-0.36	0.41
MM_0443	Phosphoribosylglycinamide formyltransferase	564674 _ 564069	0.08	0.18
MM_0444	HTH DNA-binding protein	565795 _ 564815	0.63	0.30
MM_0447	Cell division control protein (AAA family ATPase)	568898 _ 571273	0.14	0.36
MM_0448	Hypothetical protein	571422 _ 571673	-0.02	0.73
MM_0449	SAM-dependent methyltransferases	572153 _ 572860	-0.09	0.62
MM_0454	Hypothetical protein	576606 _ 576085	-0.24	0.54
MM_0455	Hypothetical protein	576755 _ 577420	-0.02	0.39
MM_0457	Recombination/repair protein RadA	579555 _ 578455	0.51	0.35
MM_0458	Cytidylyltransferase	579716 _ 580177	-0.16	0.29
MM_0459	Conserved protein	580321 _ 580650	0.31	0.35
MM_0460	Probable endonuclease IV	580792 _ 581622	0.11	0.42
MM_0462	Cobalt transport ATP-binding protein CbiO	584353 _ 582944	-0.07	0.26
MM_0463	Cobalt ABC transporter, permease protein CbiQ	585095 _ 584316	0.14	0.18
MM_0467	Conserved protein	590585 _ 587982	0.12	0.69
MM_0469	Hypothetical protein	592432 _ 593793	0.17	0.48
MM_0473	Glucokinase	598807 _ 597335	0.16	0.32
MM_0474	Glutamate N-acetyltransferase / amino-acid acetyltransferase	600358 _ 599153	0.45	0.48
MM_0475	Putative inosine-5'-monophosphate dehydrogenase	600864 _ 600358	0.13	0.16
MM_0476	N-acetyl-gamma-glutamyl-phosphate reductase	601915 _ 600896	0.05	0.50
MM_0479	Conserved protein	603626 _ 603375	0.52	0.20
MM_0481	Acetyltransferase	604311 _ 604736	-0.31	0.37
MM_0484	Hypothetical protein	608281 _ 607685	0.02	0.59
MM_0485	Phosphoglycerate kinase	609742 _ 608495	0.00	0.67
MM_0486	DNA polymerase	609943 _ 610533	-0.05	0.49
MM_0487	Heat shock protein	610856 _ 611149	-0.01	0.47
MM_0490	Phycocyanin alpha-su phycocyanobilin lyase	613161 _ 611806	0.22	0.21
MM_0494	Hypothetical protein	616158 _ 615946	0.54	0.10
MM_0495	Acetate kinase	617402 _ 616161	0.09	0.07
MM_0497	Iron-sulfur flavoprotein (Isf)	618824 _ 619399	0.11	0.31
MM_0498	Putative chloride channel protein	619683 _ 621449	-0.48	0.15
MM_0501	3-Demethylubiquinone-9 3-methyltransferase	623181 _ 623651	0.19	0.33

MM_0510	GTP-binding protein	635710 _ 634646	-0.09	0.34
MM_0511	Conserved protein	636327 _ 635935	-0.21	0.34
MM_0512	tRNA-intron endonuclease	637511 _ 636453	-0.22	0.27
MM_0514	Nitrogenase iron protein	640159 _ 639365	-0.28	0.66
MM_0515	Conserved protein	641360 _ 640251	-0.22	0.58
MM_0516	Hypothetical protein	643222 _ 641774	-0.11	0.37
MM_0517	Conserved protein	643641 _ 643252	-0.10	0.50
MM_0518	Hypothetical sensory transduction histidine kinase	646849 _ 644147	0.15	0.25
MM_0526	Hypothetical protein	654420 _ 653929	0.10	0.13
MM_0529	Hydrolase	657317 _ 656508	-0.02	0.30
MM_0530	Amino acid permease	659609 _ 657375	-0.50	0.28
MM_0531	Conserved protein	660938 _ 660582	0.08	0.54
MM_0533	Conserved protein	663713 _ 663363	-0.22	0.16
MM_0534	Conserved protein	665290 _ 664118	0.11	0.32
MM_0535	Hypothetical protein	665859 _ 665575	0.05	0.83
MM_0536	Hypothetical protein	667063 _ 666428	-0.17	0.56
MM_0537	Hypothetical protein	667417 _ 667821	0.11	0.72
MM_0542	Hypothetical protein	670830 _ 671417	0.17	0.21
MM_0544	Exoribonuclease II	672401 _ 674215	0.50	0.28
MM_0545	Hypothetical protein	674599 _ 674874	0.42	0.70
MM_0546	Conserved protein	674890 _ 675237	0.04	0.15
MM_0547	Conserved protein	675845 _ 676564	-0.02	0.38
MM_0549	Conserved protein	677325 _ 676924	0.03	0.10
MM_0552	Hypothetical protein	678858 _ 678613	0.05	0.47
MM_0555	Hypothetical protein	680804 _ 681040	-0.02	0.56
MM_0557	Hypothetical protein	683241 _ 682645	-0.07	0.67
MM_0562	Hypothetical protein	688379 _ 687684	-0.52	0.29
MM_0563	Hypothetical protein	689272 _ 688400	0.01	0.44
MM_0565	Transcriptional regulator	691202 _ 691801	0.56	0.30
MM_0566	Hypothetical protein	693250 _ 692534	0.10	0.19
MM_0567	Acetyltransferase	693485 _ 693988	0.22	0.62
MM_0570	ABC transporter, ATP-binding protein	696452 _ 695637	-0.07	0.40
MM_0573	Conserved protein	700164 _ 700661	0.03	0.66
MM_0574	Arsenate reductase	700824 _ 701273	0.23	0.41
MM_0577	Phosphate transport ATP-binding protein	704400 _ 703969	-0.12	0.32
MM_0578	Phosphate-binding protein	705393 _ 704449	0.18	0.45
MM_0579	Transposase	707283 _ 706213	0.06	0.51
MM_0580	Hypothetical protein	708299 _ 707649	-0.36	0.35
MM_0581	Conserved protein	709691 _ 708696	0.19	0.24
MM_0583	Conserved protein	711909 _ 712700	0.28	0.36
MM_0584	Conserved protein	712965 _ 713768	0.29	0.47
MM_0585	Conserved protein	714201 _ 716021	0.24	0.35
MM_0587	Conserved protein	717511 _ 718761	0.03	0.20
MM_0589	Conserved protein	720071 _ 719625	-0.06	0.10
MM_0590	Glycerol-1-phosphate dehydrogenase (putative)	721438 _ 720371	-0.04	0.68
MM_0591	Conserved membrane protein	722397 _ 723251	0.42	0.12
MM_0593	Conserved protein	724369 _ 725529	-0.41	0.27
MM_0594	Protein translation initiation factor 2 su gamma (IF-2g)	725742 _ 727034	-0.17	0.20
MM_0595	Conserved protein	727034 _ 727396	-0.12	0.42
MM_0596	DNA-directed RNA polymerase su E'	727461 _ 728042	-0.37	0.38
MM_0598	Hypothetical protein	728261 _ 728845	0.04	0.35
MM_0599	SSU ribosomal protein S24E	728829 _ 729131	-0.05	0.67
MM_0602	O-sialoglycoprotein endopeptidase	733754 _ 735439	-0.18	0.33
MM_0603	Nucleoside-triphosphatase	735435 _ 735986	-0.35	0.35
MM_0606	Hypothetical protein	738099 _ 737374	0.37	0.53
MM_0607	Ferredoxin	738368 _ 738210	0.51	0.34
MM_0608	Probable radical-forming protein	738541 _ 739599	-0.02	0.19
MM_0609	Conserved protein	739817 _ 740401	-0.21	0.28
MM_0610	Acetyltransferases	741532 _ 740972	0.11	0.58
MM_0612	NH3-dependent NAD(+) synthetase	744037 _ 742976	-0.16	0.34

MM_0613	Conserved protein	745310 _ 744276	-0.08	0.17
MM_0614	Long-chain-fatty-acid--CoA ligase	746778 _ 745294	0.15	0.58
MM_0617	Conserved protein	748892 _ 749158	-0.25	0.40
MM_0618	Undecaprenyl pyrophosphate synthetase	749737 _ 750708	0.34	0.59
MM_0619	Conserved protein	751213 _ 753312	-0.45	0.60
MM_0620	Conserved protein	753388 _ 753639	0.35	0.54
MM_0621	Hypothetical protein	753823 _ 754266	0.36	0.24
MM_0622	Transposase	754286 _ 754792	-0.23	0.52
MM_0624	Hypothetical protein	755503 _ 756231	0.11	0.53
MM_0626	Nicotinamide-nucleotide adenylyltransferase	757608 _ 757066	0.26	0.51
MM_0627	F420H2 dehydrogenase, su FpoF	758713 _ 757676	0.01	0.47
MM_0628	Coenzyme F420-dependent N5,N10-methylene tetrahydromethanopterin reductase	759801 _ 758818	-0.11	0.48
MM_0629	Zinc finger protein	760739 _ 760966	0.35	0.31
MM_0630	Zinc finger protein	760966 _ 761199	0.21	0.50
MM_0631	Hypothetical protein similar to COX locus protein	761401 _ 761745	0.23	0.22
MM_0632	Desulfoferrodoxin	761952 _ 762434	0.18	0.34
MM_0633	Cytochrome c	762520 _ 763851	0.50	0.41
MM_0634	Iron-sulfur flavoprotein	764022 _ 764705	0.15	0.35
MM_0635	Flavoprotein	764813 _ 765445	-0.13	0.40
MM_0636	Conserved protein	765497 _ 766000	0.10	0.58
MM_0637	Putative flavodoxin	766027 _ 766233	0.13	0.52
MM_0640	Hypothetical protein	768615 _ 768854	0.07	0.42
MM_0641	Conserved protein	769215 _ 768949	0.00	0.58
MM_0642	Isocitrate dehydrogenase [NADP]	770263 _ 769274	-0.01	0.36
MM_0643	Conserved protein	770889 _ 770332	-0.04	0.19
MM_0644	Conserved protein	771862 _ 770975	0.00	0.21
MM_0645	3-Isopropylmalate dehydratase	772490 _ 771984	0.14	0.40
MM_0646	Oligosaccharyl transferase	775174 _ 772655	0.11	0.61
MM_0647	Oligosaccharyl transferase	778053 _ 775510	-0.05	0.19
MM_0648	Glycosyl transferase	779245 _ 778094	0.24	0.41
MM_0649	Glycosyl transferase	779325 _ 780446	-0.12	0.38
MM_0650	Mannosyltransferase	780497 _ 781555	-0.11	0.37
MM_0651	Glycosyltransferase involved in cell wall biogenesis	782583 _ 781558	-0.16	0.75
MM_0652	Glycosyltransferase involved in cell wall biogenesis	783503 _ 782583	-0.30	0.45
MM_0653	Glycosyltransferase involved in cell wall biogenesis	784502 _ 783759	-0.47	0.46
MM_0654	Mannosyltransferase	785629 _ 784520	-0.27	0.40
MM_0656	Polysaccharide ABC transporter, ATP-binding protein	787431 _ 786169	-0.28	0.35
MM_0658	GDP-fucose synthetase	789325 _ 788390	-0.12	0.59
MM_0659	GDP-mannose 4,6 dehydratase	790398 _ 789346	-0.10	0.37
MM_0660	Mannose-6-phosphate isomerase/ mannose-1-phosphate guanylyl transferase	791796 _ 790492	-0.10	0.22
MM_0662	Transcriptional regulator, ArsR family	793386 _ 792970	0.11	0.40
MM_0663	Probable dihydroorotate dehydrogenase electron transfer su	794499 _ 795341	-0.46	0.36
MM_0664	Glutamate synthase [NADPH]	795363 _ 796769	-0.21	0.43
MM_0666	Putative nickel-responsive regulator NikR	799088 _ 799525	0.28	0.77
MM_0667	Nitroreductase family protein	800868 _ 800329	0.11	0.53
MM_0668	Ketol-acid reductoisomerase	802135 _ 801131	0.13	0.19
MM_0669	Acetolactate synthase small su	803014 _ 802532	0.65	0.19
MM_0670	Acetolactate synthase large su	804705 _ 803014	0.48	0.24
MM_0671	2-Isopropylmalate synthase	806460 _ 805012	0.39	0.49
MM_0672	Hypothetical protein	807798 _ 807100	-0.15	0.64
MM_0673	Conserved protein	810028 _ 808946	0.25	0.35
MM_0674	Prefoldin beta su	810703 _ 811053	0.24	0.55
MM_0675	Conserved protein	811179 _ 812180	0.13	0.28
MM_0676	Coenzyme F390 synthetase/phenylacetyl-CoA ligase	812578 _ 813888	0.03	0.52
MM_0677	Acetolactate synthase small su	814249 _ 814695	0.18	0.45
MM_0678	Conserved protein	815015 _ 815365	0.03	0.33
MM_0679	Conserved protein	815389 _ 816363	-0.25	0.55
MM_0680	Conserved protein	817001 _ 816411	0.46	0.33

MM_0683	Hypothetical protein	818872 _ 818642	0.50	0.21
MM_0687	Nickel-insertion protein CooH	824362 _ 825120	0.11	0.53
MM_0690	Conserved protein	828728 _ 829789	-0.01	0.47
MM_0691	Hypothetical protein	829810 _ 831006	-0.07	0.56
MM_0692	Transcription initiation factor IIE, alpha su	831128 _ 831619	-0.42	0.18
MM_0693	Conserved protein	831633 _ 832142	-0.15	0.39
MM_0694	Proteasome, beta su	832393 _ 833022	-0.14	0.59
MM_0695	Cleavage and polyadenylation specificity factor, 100 kD su	833137 _ 835047	-0.23	0.88
MM_0697	Cell division protein	836856 _ 838031	0.02	0.42
MM_0698	Dihydropteroate synthase	838235 _ 839713	0.16	0.44
MM_0699	Conserved protein	839754 _ 840209	0.17	0.32
MM_0700	Vacuolar-type H+-pyrophosphatase	842992 _ 840965	-0.17	0.38
MM_0701	Vacuolar-type H+-pyrophosphatase	843903 _ 845915	0.16	0.26
MM_0702	Hypothetical permease	846843 _ 847973	-0.06	0.39
MM_0704	Conserved protein	849409 _ 849891	-0.19	0.42
MM_0705	Conserved protein	850069 _ 850446	0.10	0.48
MM_0706	ZPR1-related zinc finger protein	850446 _ 851087	0.18	0.51
MM_0707	Prolyl-tRNA synthetase	851567 _ 853006	0.07	0.44
MM_0708	Conserved protein	853539 _ 854585	-0.40	0.30
MM_0709	Conserved protein	855057 _ 854845	0.01	0.63
MM_0710	Hypothetical protein	855588 _ 855307	-0.02	0.45
MM_0711	Transposase	856718 _ 858145	0.30	0.38
MM_0712	Hypothetical protein	858345 _ 859100	0.04	0.40
MM_0713	Conserved protein	859100 _ 859885	-0.09	0.47
MM_0714	Fructose-bisphosphate aldolase	860242 _ 861168	0.14	0.32
MM_0715	Pyruvate kinase	861276 _ 862706	-0.13	0.21
MM_0716	Hypothetical protein	862919 _ 863833	0.51	0.50
MM_0717	Hypothetical protein	864124 _ 864447	0.62	0.21
MM_0718	Oxidoreductase (flavoprotein)	865934 _ 864711	0.27	0.33
MM_0719	Nitrogenase iron protein	866092 _ 866910	-0.14	0.74
MM_0722	Nitrogenase molybdenum-iron protein alpha chain	867704 _ 869299	0.00	0.69
MM_0723	Nitrogenase molybdenum-iron protein beta chain	869316 _ 870683	-0.19	0.27
MM_0725	Nitrogenase iron-molybdenum cofactor biosynthesis protein NifN	872472 _ 874049	0.17	0.32
MM_0726	Molybdate-binding protein	874049 _ 874864	0.14	0.34
MM_0728	Molybdenum transporter, ATP-binding protein	875654 _ 876778	0.09	0.58
MM_0730	SAM-dependent methyltransferases	881011 _ 880283	0.26	0.32
MM_0733	Ammonium transporter (Amt1)	881714 _ 882910	0.15	0.26
MM_0736	Transporter	884177 _ 885208	0.13	0.55
MM_0737	Conserved protein	885319 _ 885558	0.01	0.18
MM_0738	Conserved protein	885592 _ 885972	-0.06	0.71
MM_0739	Transposase	887295 _ 886165	-0.05	0.50
MM_0741	Universal stress protein	887928 _ 888812	0.22	0.62
MM_0742	Arsenical-resistance protein	888778 _ 889857	0.16	0.27
MM_0744	Conserved protein	891985 _ 890399	0.53	0.39
MM_0745	Conserved protein	892895 _ 892200	-0.43	0.38
MM_0746	Conserved protein	894073 _ 894495	0.28	0.09
MM_0747	ATP-dependent RNA helicase	895027 _ 896787	-0.78	0.20
MM_0748	Conserved protein	897586 _ 896888	0.14	0.33
MM_0749	Glutathione-independent formaldehyde dehydrogenase	899330 _ 897993	-0.10	0.11
MM_0751	Putative serine/threonine protein phosphatase	901679 _ 902377	-0.09	0.40
MM_0753	Conserved protein	903166 _ 903858	-0.58	0.27
MM_0754	Conserved protein	903977 _ 904534	-0.44	0.22
MM_0756	6-Pyruvoyltetrahydropterin synthase	905327 _ 905674	-0.41	0.34
MM_0757	NifB protein	906242 _ 907225	0.11	0.30
MM_0760	Ferredoxin	908924 _ 908751	-0.25	0.55
MM_0761	Thiamin-monophosphate kinase	909410 _ 910387	-0.31	0.37
MM_0762	Conserved protein	910811 _ 910512	-0.17	0.52
MM_0764	Conserved protein	912211 _ 912618	0.21	0.71
MM_0765	Conserved protein	913971 _ 913009	-0.13	0.19

MM_0766	Transposase	915277 _ 914180	0.44	0.28
MM_0767	Conserved protein	916182 _ 915820	-0.10	0.28
MM_0768	Glycosyl transferase	916716 _ 917648	-0.24	0.33
MM_0770	Conserved protein	919540 _ 920361	-0.40	0.31
MM_0772	Conserved protein	922435 _ 922839	-0.91	0.07
MM_0774	Conserved protein	924766 _ 924362	-0.13	0.55
MM_0775	Conserved protein	925877 _ 927358	-0.08	0.26
MM_0778	A1AO H+ ATPASE, su D	931634 _ 930999	-0.19	0.23
MM_0779	A1AO H+ ATPASE, su B	933067 _ 931634	0.18	0.66
MM_0780	A1AO H+ ATPASE, su A	934751 _ 933018	0.02	0.61
MM_0781	A1AO H+ ATPASE, su F	935047 _ 934745	0.02	0.68
MM_0782	A1AO H+ ATPASE, su C	936129 _ 935050	-0.03	0.47
MM_0783	A1AO H+ ATPASE, su E	936687 _ 936139	0.01	0.66
MM_0784	A1AO H+ ATPASE, su K	937138 _ 936899	0.14	0.48
MM_0785	A1AO H+ ATPASE, su I	939110 _ 937146	-0.09	0.71
MM_0786	A1AO H+ ATPASE, su H	939357 _ 939088	-0.14	0.51
MM_0788	Metallo cofactor biosynthesis protein moaA/ nifB /pqqE family	942609 _ 941404	-0.06	0.86
MM_0789	Geranyltranstransferase/Farnesyltranstransferase/ Hexaprenyl diphosphate synthase	943752 _ 942868	0.19	0.37
MM_0790	Hypothetical protein	944087 _ 943731	-0.04	0.41
MM_0792	Hypothetical protein	947277 _ 945751	0.58	0.17
MM_0794	ADP-ribosylglycohydrolase	948872 _ 947886	0.12	0.65
MM_0795	Hypothetical protein	949516 _ 949124	-0.34	0.23
MM_0797	Hypothetical protein	951126 _ 951692	0.22	0.45
MM_0798	26S Proteasome regulatory su RPT2/S4	953293 _ 952055	0.11	0.75
MM_0799	Hypothetical protein	954184 _ 954426	0.46	0.57
MM_0800	Hypothetical protein	954552 _ 954782	-0.06	0.68
MM_0801	Adenylosuccinate synthetase	954914 _ 956050	-0.21	0.31
MM_0802	SSU ribosomal protein S19E	956480 _ 956926	-0.05	0.25
MM_0803	Double-stranded DNA-binding protein	957016 _ 957375	-0.31	0.19
MM_0804	Conserved hypothetical protein	957384 _ 957983	-0.17	0.35
MM_0805	LSU ribosomal protein L39E	958265 _ 958405	0.02	0.31
MM_0806	LSU ribosomal protein L31E	958415 _ 958693	-0.35	0.29
MM_0807	Protein translation initiation factor 6 (IF-6)	958725 _ 959381	-0.29	0.48
MM_0809	Prefoldin, alpha su	959610 _ 960044	-0.16	0.31
MM_0810	Signal recognition particle, su Ffh/SRP54	960228 _ 961484	-0.38	0.38
MM_0811	Stress-responsive transcriptional regulator	961960 _ 961784	-0.18	0.47
MM_0813	Conserved protein	964313 _ 963333	0.14	0.48
MM_0814	Probable peroxiredoxin	964752 _ 965408	0.08	0.27
MM_0815	Transposase (N-terminal domain)	965530 _ 965835	0.37	0.19
MM_0817	Pyrroline-5-carboxylate reductase	968465 _ 967656	0.24	0.50
MM_0818	Glutamate 5-kinase	969803 _ 968649	-0.33	0.19
MM_0819	Gamma-glutamyl phosphate reductase	971284 _ 969944	-0.10	0.30
MM_0820	Conserved protein	972172 _ 972573	0.00	0.55
MM_0821	Conserved protein	973135 _ 972686	0.12	0.45
MM_0823	Conserved protein	974188 _ 975294	0.14	0.29
MM_0824	Universal stress protein	975317 _ 975772	-0.02	0.58
MM_0825	Conserved protein	975846 _ 976694	0.20	0.37
MM_0826	Conserved protein	976865 _ 977548	-0.19	0.38
MM_0827	Conserved protein	978390 _ 977674	-0.25	0.22
MM_0828	Conserved protein	979613 _ 979248	-0.22	0.05
MM_0829	Conserved protein	979997 _ 979620	-0.21	0.12
MM_0830	Molybdopterin biosynthesis MoeA protein	981326 _ 980139	0.17	0.16
MM_0832	Conserved protein	981722 _ 983305	0.13	0.04
MM_0834	Na+/H+ antiporter	984394 _ 985536	-0.15	0.27
MM_0835	Cation-transporting ATPase	988448 _ 985719	0.19	0.49
MM_0836	Conserved protein	988734 _ 990062	-0.01	0.34
MM_0838	Succinate-semialdehyde dehydrogenase [NADP+]	992397 _ 990961	-0.14	0.55
MM_0840	Glycosyltransferases involved in cell wall biogenesis (putative)	995647 _ 997098	-0.10	0.28

MM_0841	Transcriptional regulator, ArsR family	997349 _ 997711	0.18	0.35
MM_0842	Conserved protein	998315 _ 997815	0.30	0.19
MM_0843	Coenzyme F390 synthetase/phenylacetyl-CoA ligase	999921 _ 998566	0.60	0.35
MM_0844	Hypothetical protein	1000618 _ 1000226	0.29	0.60
MM_0845	Conserved protein	1000966 _ 1000640	0.70	0.16
MM_0846	Conserved protein	1001601 _ 1001008	-0.28	0.21
MM_0849	Bacterioferritin comigratory protein	1003540 _ 1004019	0.47	0.33
MM_0851	Conserved protein	1005200 _ 1004838	-0.01	0.54
MM_0853	Conserved protein	1007855 _ 1007118	-0.05	0.49
MM_0854	Cell division cycle protein 48 homolog	1009138 _ 1007855	0.05	0.26
MM_0855	Phosphoribosylamidoimidazole-succinocarboxamide synthase	1009614 _ 1010324	0.13	0.29
MM_0856	Conserved protein	1011800 _ 1010577	-0.28	0.15
MM_0857	Conserved protein	1012162 _ 1011800	0.41	0.33
MM_0858	Protein translation initiation factor 1 (IF-1)	1012669 _ 1012364	0.02	0.04
MM_0859	Conserved protein	1013221 _ 1013457	0.51	0.31
MM_0860	Phosphoribosylformylglycinamidine synthase	1014013 _ 1016004	0.13	0.48
MM_0861	Alpha-amylase	1016498 _ 1017631	0.10	0.20
MM_0862	Alpha-amylase	1017631 _ 1018824	0.26	0.48
MM_0863	Conserved protein	1018878 _ 1019681	0.03	0.57
MM_0865	Seryl-tRNA synthetase	1023136 _ 1021871	-0.01	0.58
MM_0866	Periplasmic serine protease	1024490 _ 1023261	-0.11	0.61
MM_0868	Adenylate cyclase	1027713 _ 1027186	0.49	0.46
MM_0869	Conserved protein	1028225 _ 1027830	0.49	0.37
MM_0870	Beta-ketoacyl synthase/ thiolase	1029404 _ 1028235	0.36	0.51
MM_0871	Hydroxymethylglutaryl-CoA synthase	1030471 _ 1029425	0.21	0.28
MM_0872	Putative transcriptional regulator	1031252 _ 1030515	0.29	0.30
MM_0873	Conserved protein	1031391 _ 1032209	0.26	0.17
MM_0874	Conserved protein	1032667 _ 1033416	-0.26	0.60
MM_0876	Hypothetical protein	1035461 _ 1036195	-0.05	0.62
MM_0877	Transposase	1036566 _ 1036895	1.04	0.20
MM_0880	Conserved protein	1038618 _ 1039394	-0.44	0.26
MM_0881	Transposase	1041288 _ 1039987	-0.03	0.35
MM_0882	Conserved protein	1041468 _ 1042397	0.11	0.64
MM_0883	Conserved protein	1042702 _ 1044603	-0.31	0.26
MM_0884	Conserved protein	1045021 _ 1044770	0.41	0.47
MM_0886	DNA polymerase IV	1046680 _ 1045586	0.37	0.54
MM_0887	Cobalt transport ATP-binding protein CbiO	1046973 _ 1047953	0.03	0.23
MM_0888	Cobalt ABC transporter, permease protein CbiQ	1048009 _ 1048812	0.07	0.27
MM_0889	Hypothetical protein	1050470 _ 1048890	-0.12	0.51
MM_0892	Hypothetical protein	1053879 _ 1053058	0.25	0.61
MM_0893	Cobalamin biosynthesis protein CbiM	1054360 _ 1055022	0.26	0.64
MM_0894	Hypothetical protein	1055022 _ 1055324	0.23	0.47
MM_0895	Endonuclease III	1056185 _ 1055484	0.32	0.29
MM_0897	Glutathione reductase	1057686 _ 1056607	-0.19	0.31
MM_0898	Bifunctional purine biosynthesis protein PurH	1058082 _ 1059695	0.08	0.18
MM_0899	Conserved protein	1060415 _ 1061197	0.18	0.46
MM_0901	Cation-transporting ATPase	1063538 _ 1066372	0.38	0.68
MM_0902	Putative methyltransferase	1067254 _ 1066418	0.12	0.23
MM_0904	Phosphoglycerate mutase	1068439 _ 1070001	-0.34	0.35
MM_0905	Conserved protein	1070316 _ 1071269	0.10	0.63
MM_0906	Flap endonuclease-1 (RAD27/FEN1 family)	1072828 _ 1071815	0.32	0.60
MM_0907	Hypothetical protein	1073738 _ 1073439	0.28	0.41
MM_0909	Conserved protein	1075602 _ 1076492	0.01	0.28
MM_0910	Potassium/copper-transporting ATPase	1076614 _ 1077411	-0.15	0.43
MM_0911	ABC transporter, ATP-binding protein	1077662 _ 1079272	-0.06	0.45
MM_0912	Conserved protein	1079317 _ 1080891	0.18	0.31
MM_0913	Conserved protein	1081063 _ 1081503	0.07	0.09
MM_0914	Conserved protein	1081503 _ 1082012	-0.13	0.37
MM_0915	Conserved protein	1082012 _ 1083256	-0.10	0.39

MM_0916	Conserved protein	1083267 _ 1083863	-0.07	0.45
MM_0917	Conserved protein	1083868 _ 1084800	0.05	0.26
MM_0918	4-Carboxymuconolactone decarboxylase	1084945 _ 1085439	-0.08	0.47
MM_0919	Conserved protein	1085885 _ 1086544	-0.52	0.10
MM_0920	MutT related protein	1087018 _ 1086584	-0.17	0.35
MM_0922	Protein iranslation initiation factor 5A (IF-5A)	1089329 _ 1089712	-0.33	0.41
MM_0923	Agmatinase	1089822 _ 1090694	-0.31	0.09
MM_0924	Conserved protein	1091157 _ 1090984	0.37	0.31
MM_0931	Hypothetical protein	1098506 _ 1097778	-0.17	0.27
MM_0933	Glutathione-regulated potassium-efflux system protein	1101538 _ 1099529	0.09	0.67
MM_0935	Beta-lysine acetyltransferase	1103966 _ 1104778	-0.10	0.25
MM_0936	Conserved protein	1105420 _ 1104806	-0.18	0.38
MM_0939	Putative flagella-related protein FlaH	1111083 _ 1110370	0.12	0.17
MM_0940	Putative flavodoxin	1112100 _ 1113725	0.31	0.51
MM_0941	Adenylosuccinate lyase	1116454 _ 1115099	0.23	0.19
MM_0942	Hypothetical protein	1117018 _ 1116758	-0.20	0.42
MM_0943	Geranylgeranylglyceryl diphosphate synthase	1117122 _ 1117862	0.13	0.24
MM_0944	Methyl coenzyme M reductase, component A2 homolog	1118334 _ 1120094	0.01	0.39
MM_0949	Hypothetical protein	1126439 _ 1125186	-0.26	0.24
MM_0950	ABC transporter, ATP-binding protein	1127469 _ 1126591	-0.17	0.36
MM_0954	Hypothetical protein	1132202 _ 1131816	-0.18	0.29
MM_0960	Small heat shock protein	1140024 _ 1140320	-0.24	0.15
MM_0961	Putative small heat shock protein	1140391 _ 1140696	-0.15	0.42
MM_0962	Conserved protein	1140845 _ 1141069	0.02	0.58
MM_0963	Integral Membrane protein	1141824 _ 1141315	0.54	0.33
MM_0966	Glutamate synthase, large chain	1144225 _ 1145319	-0.48	0.37
MM_0967	Glutamate synthase, large chain	1145319 _ 1146827	-0.52	0.30
MM_0968	Glutamate synthase, large chain	1146833 _ 1147573	-0.27	0.27
MM_0969	F420 hydrogenase/dehydrogenase, beta su	1147583 _ 1148614	-0.03	0.64
MM_0970	Hypothetical protein	1149665 _ 1148793	-0.16	0.56
MM_0971	Hypothetical protein	1150597 _ 1149722	0.20	0.31
MM_0972	Conserved protein	1151247 _ 1151480	-0.21	0.10
MM_0974	Xaa-Pro aminopeptidase	1155210 _ 1154011	0.19	0.31
MM_0975	Conserved protein	1155475 _ 1156011	0.06	0.37
MM_0976	Protease (putative)	1157836 _ 1156106	0.09	0.17
MM_0978	Hypothetical protein	1159051 _ 1159278	-0.20	0.17
MM_0979	Heterodisulfate reductase, su HdrB	1159538 _ 1160020	-0.19	0.34
MM_0980	Heterodisulfate reductase, su HdrB	1160001 _ 1160936	-0.13	0.25
MM_0981	Iron-sulfur binding protein	1161411 _ 1160986	-0.17	0.29
MM_0982	Transposase (N-terminal domain)	1161850 _ 1161545	-0.03	0.52
MM_0983	Conserved protein	1162567 _ 1161962	-0.17	0.39
MM_0984	Conserved protein	1163377 _ 1162685	0.07	0.21
MM_0985	Conserved protein	1164530 _ 1163589	0.13	0.33
MM_0987	Conserved protein	1165792 _ 1165430	-0.06	0.19
MM_0988	Conserved protein	1166252 _ 1165890	0.10	0.51
MM_0989	Conserved protein	1166462 _ 1166821	0.05	0.43
MM_0990	Nucleotide-binding protein	1166808 _ 1167713	-0.02	0.37
MM_0991	Thiol-disulfide isomerase/thioredoxin	1168022 _ 1168516	-0.01	0.54
MM_0994	Precorrin-8X methylmutase	1170905 _ 1170177	0.14	0.73
MM_0995	Precorrin-3B C17-methyltransferase	1171692 _ 1170898	-0.04	0.70
MM_0996	Cobalamin biosynthesis protein CbiG	1172032 _ 1171664	-0.23	0.40
MM_0998	Precorrin-4 C11-methyltransferase	1173358 _ 1172633	-0.12	0.36
MM_0999	Precorrin-2 C20-methyltransferase	1174145 _ 1173540	-0.24	0.50
MM_1000	Precorrin-6Y C5,15-methyltransferase [decarboxylating]	1174790 _ 1174239	-0.24	0.32
MM_1001	Hypothetical protein	1175586 _ 1175371	0.07	0.28
MM_1002	2-Isopropylmalate synthase	1175568 _ 1176887	-0.22	0.43
MM_1003	Isocitrate dehydrogenase [NADP]	1178084 _ 1177059	0.07	0.64
MM_1004	Putative molybdenum cofactor biosynthesis protein	1178389 _ 1179228	-0.10	0.20
MM_1005	HTH DNA-binding protein	1180064 _ 1179582	0.27	0.55
MM_1006	26S Proteasome regulatory su RPT2/S5	1180095 _ 1181414	0.23	0.51

MM_1007	Conserved protein	1181553 _ 1182140	0.09	0.50
MM_1008	Cell division protein	1182688 _ 1183809	0.27	0.54
MM_1009	Protein translocase, su SecE	1184237 _ 1184449	-0.17	0.29
MM_1010	Putative transcription antitermination protein nusG	1184437 _ 1184904	-0.35	0.41
MM_1012	LSU ribosomal protein L1P	1185755 _ 1186393	-0.22	0.44
MM_1013	LSU ribosomal protein L10P	1186397 _ 1187437	-0.11	0.31
MM_1014	LSU ribosomal protein L12AE	1187483 _ 1187794	-0.14	0.27
MM_1015	Molybdenum cofactor biosynthesis enzyme (Fe-S oxidoreductase family)	1188086 _ 1189216	0.06	0.55
MM_1017	Conserved protein	1191451 _ 1190666	0.51	0.17
MM_1019	Iron-sulfur cluster-binding protein	1192926 _ 1192186	-0.08	0.31
MM_1023	Conserved protein	1195438 _ 1195653	-0.16	0.20
MM_1024	Conserved protein	1195815 _ 1196915	0.24	0.46
MM_1025	Thiamine biosynthesis protein	1198363 _ 1197059	-0.28	0.55
MM_1026	Dolichol-phosphate mannosyltransferase	1199673 _ 1198759	-0.33	0.53
MM_1027	Transcription initiation factor TFIIB	1200349 _ 1199801	0.12	0.52
MM_1028	Transcription initiation factor TFIIB	1200980 _ 1200432	-0.08	0.33
MM_1030	Conserved protein	1203339 _ 1202338	0.02	0.31
MM_1031	Chromosome partition protein	1206859 _ 1203335	0.11	0.47
MM_1032	Conserved protein	1207879 _ 1206914	0.11	0.48
MM_1033	Ferrous iron transport protein B	1211098 _ 1209104	-0.01	0.48
MM_1034	Transcriptional repressor	1211812 _ 1211162	0.10	0.36
MM_1035	Amino-acid acetyltransferase	1212528 _ 1212052	0.15	0.22
MM_1036	BioY protein	1213256 _ 1213816	-0.03	0.41
MM_1037	Cobalt transport ATP-binding protein CbiO	1213905 _ 1214726	-0.11	0.48
MM_1038	Cobalt transport ATP-binding protein CbiO	1214663 _ 1215565	-0.32	0.21
MM_1039	Cobalt ABC transporter, permease protein CbiQ	1215639 _ 1216409	-0.01	0.20
MM_1040	Predicted transcriptional regulator	1216520 _ 1216864	0.31	0.40
MM_1045	Branched-chain amino acid aminotransferase	1220896 _ 1221777	-0.17	0.58
MM_1046	Molybdopterin biosynthesis protein	1222217 _ 1224208	0.18	0.31
MM_1047	GTP-binding protein	1224590 _ 1225681	-0.07	0.32
MM_1048	D-alanine-D-alanine ligase related protein	1228016 _ 1225917	-0.17	0.24
MM_1049	Arsenite permease	1229469 _ 1228210	0.00	0.31
MM_1051	Chromosomal protein MC1	1230382 _ 1230101	-0.26	0.09
MM_1052	Conserved protein	1230607 _ 1230858	0.14	0.61
MM_1053	Putative Sensory protein	1232967 _ 1234535	-0.01	0.67
MM_1055	Trimethylamine corrionid protein	1237168 _ 1237698	-0.08	0.18
MM_1056	Putative ATP-dependent Na+ efflux pump	1239742 _ 1238468	0.18	0.40
MM_1057	ABC transporter, ATP-binding protein	1240622 _ 1239714	0.15	0.51
MM_1058	Diaminohydroxyphosphoribosylaminopyrimidine reductase	1240733 _ 1241401	-0.19	0.18
MM_1059	Formate hydrogenlyase su 3	1242273 _ 1244201	-0.11	0.07
MM_1060	Formate hydrogenlyase su 4	1244201 _ 1245139	-0.39	0.60
MM_1061	Hydrogenase-4 component E	1245139 _ 1245822	-0.16	0.41
MM_1063	Formate hydrogenlyase su 5 precursor	1247405 _ 1248994	-0.28	0.23
MM_1064	Formate hydrogenlyase su 7	1249083 _ 1249592	0.23	0.34
MM_1067	Conserved protein	1250650 _ 1251432	0.32	0.50
MM_1068	Two-component response regulator	1252203 _ 1251835	0.51	0.33
MM_1071	Conserved protein	1257153 _ 1258769	-0.15	0.63
MM_1072	Conserved protein	1259208 _ 1260362	-0.42	0.72
MM_1073	Methanol corrinoid protein MtaC2	1262243 _ 1263016	0.21	0.46
MM_1076	Cation efflux protein	1265444 _ 1266433	-0.11	0.85
MM_1077	Conserved protein	1266832 _ 1268844	0.13	0.51
MM_1078	Conserved protein	1268995 _ 1270920	0.03	0.52
MM_1079	Conserved protein	1271052 _ 1271834	0.01	0.53
MM_1080	Conserved protein	1272189 _ 1272974	-0.01	0.44
MM_1081	Conserved protein	1273541 _ 1273164	-0.09	0.48
MM_1082	GTP-binding protein	1274097 _ 1275278	-0.21	0.34
MM_1083	Undecaprenyl pyrophosphate synthetase	1275386 _ 1276057	-0.05	0.65
MM_1085	Conserved protein	1277375 _ 1278343	0.15	0.56
MM_1087	ABC transporter, ATP-binding protein	1279667 _ 1280407	0.05	0.39

MM_1088	ABC transporter, ATP-binding protein	1280392 _ 1281612	-0.07	0.67
MM_1089	Putative DNA integration/recombination/invertion protein	1282330 _ 1282632	0.14	0.20
MM_1092	Thiol-disulfide isomerase/thioredoxin related protein	1284655 _ 1285026	-0.28	0.08
MM_1093	Hypothetical sensory transduction histidine kinase	1285023 _ 1287515	-0.42	0.35
MM_1094	Putative transcriptional regulator	1288279 _ 1288638	-0.09	0.49
MM_1096	Thermosome, gamma su	1289933 _ 1291558	0.09	0.57
MM_1097	Conserved protein	1292317 _ 1291931	0.16	0.16
MM_1098	Conserved protein	1293071 _ 1292325	0.07	0.25
MM_1100	Hypothetical protein	1293731 _ 1294315	0.07	0.18
MM_1101	Archaeosine tRNA-ribosyltransferase	1294441 _ 1295910	-0.07	0.52
MM_1103	Hypothetical protein	1296492 _ 1297145	0.02	0.27
MM_1104	Oxidoreductase (flavoprotein)	1297177 _ 1298331	0.04	0.13
MM_1105	Metallo cofactor biosynthesis protein moaA/ nifB /pqqE family	1298445 _ 1299632	-0.13	0.53
MM_1106	Putative phosphoserine phosphatase	1300790 _ 1299936	-0.04	0.46
MM_1107	Putative phosphoserine phosphatase	1301538 _ 1300834	-0.49	0.45
MM_1108	F420-dependent methylenetetrahydromethanopterin dehydrogenase	1301932 _ 1302768	0.34	0.32
MM_1109	Conserved protein	1303380 _ 1302973	0.37	0.34
MM_1110	Conserved protein	1304134 _ 1303475	0.29	0.45
MM_1111	Thymidylate kinase	1304292 _ 1304909	-0.07	0.33
MM_1113	Phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	1306223 _ 1305504	0.20	0.66
MM_1114	Conserved protein	1306336 _ 1307037	0.27	0.70
MM_1115	Conserved protein	1307149 _ 1307685	0.23	0.52
MM_1116	Hypothetical protein	1309862 _ 1308234	0.16	0.24
MM_1117	Polysaccharide deacetylase	1311627 _ 1310725	-0.14	0.37
MM_1118	Conserved protein	1313567 _ 1315486	-0.09	0.48
MM_1119	Conserved protein	1316062 _ 1317669	-0.09	0.48
MM_1120	Conserved protein	1318036 _ 1320129	0.19	0.49
MM_1121	Glycosyl transferase	1321992 _ 1320400	-0.11	0.52
MM_1122	Oxidoreductase (hypothetical)	1323227 _ 1324174	0.32	0.47
MM_1123	Hypothetical protein	1324827 _ 1326767	-0.10	0.44
MM_1124	O-antigen translocase	1326888 _ 1328342	-0.17	0.39
MM_1125	Conserved protein	1329509 _ 1328505	-0.20	0.28
MM_1127	Glycosyl transferase	1331770 _ 1330847	-0.12	0.81
MM_1128	Conserved protein	1332809 _ 1331763	-0.20	0.24
MM_1129	Conserved protein	1333887 _ 1332796	0.13	0.43
MM_1130	Hypothetical protein	1334146 _ 1333865	0.12	0.53
MM_1132	UDP-glucose 6-dehydrogenase	1335013 _ 1336263	0.01	0.21
MM_1133	UTP--glucose-1-phosphate uridylyltransferase	1337356 _ 1336388	0.19	0.52
MM_1134	UDP-glucose 4-epimerase	1337476 _ 1338423	0.29	0.17
MM_1135	Transporter	1340172 _ 1338697	-0.10	0.37
MM_1137	Glycosyl transferase	1346085 _ 1344904	0.11	0.17
MM_1138	Glycosyl transferase	1346281 _ 1347483	-0.11	0.32
MM_1139	Dolichyl-phosphate mannose synthase related protein	1349084 _ 1348068	-0.11	0.55
MM_1140	Dolichyl-phosphate mannose synthase related protein	1350005 _ 1349109	-0.19	0.56
MM_1141	Glycosyl transferase	1350918 _ 1351850	-0.03	0.43
MM_1142	Glycosyl transferase	1351948 _ 1353084	-0.27	0.37
MM_1143	Glycosyl transferase	1353221 _ 1354399	-0.33	0.41
MM_1144	Disaggregase	1355331 _ 1358615	-0.44	0.29
MM_1145	Conserved protein	1360337 _ 1358733	-0.21	0.50
MM_1147	Dolichol-phosphate mannosyltransferase	1364042 _ 1363149	-0.16	0.33
MM_1148	Conserved protein	1364934 _ 1365827	0.05	0.24
MM_1149	Conserved protein	1365833 _ 1366687	0.21	0.24
MM_1150	Conserved protein	1367197 _ 1367006	-0.04	0.23
MM_1151	Acetyltransferase	1368204 _ 1368869	-0.02	0.42
MM_1152	Aspartate aminotransferase	1368934 _ 1370013	-0.05	0.39
MM_1153	Myo-inositol 2-dehydrogenase	1370062 _ 1370997	-0.30	0.29
MM_1154	NDP-N-acetyl-D-galactosaminuronic acid dehydrogenase	1371087 _ 1372520	0.00	0.13
MM_1155	Conserved protein	1372578 _ 1373621	0.15	0.68

MM_1156	O-antigen translocase	1374221 _ 1375726	-0.05	0.40
MM_1157	Conserved protein	1376323 _ 1377006	-0.18	0.48
MM_1158	Conserved protein	1377466 _ 1378812	0.06	0.59
MM_1159	Hypothetical protein	1379777 _ 1379079	0.03	0.64
MM_1160	Nucleotidyltransferase	1380238 _ 1380666	0.01	0.17
MM_1161	Conserved protein	1380666 _ 1381085	0.27	0.50
MM_1162	UDP-N-acetylglucosamine 4-epimerase	1382250 _ 1381249	0.04	0.44
MM_1164	Putative nucleotidyltransferase	1383407 _ 1383763	0.12	0.54
MM_1165	Putative glycosyl hydrolase	1384695 _ 1383862	0.07	0.32
MM_1166	dTDP-4-dehydrorhamnose 3,5-epimerase	1385394 _ 1385942	0.26	0.50
MM_1168	dTDP-4-dehydrorhamnose reductase	1387096 _ 1387902	-0.37	0.63
MM_1169	Glucose-1-phosphate thymidylyltransferase	1387902 _ 1388186	-0.15	0.64
MM_1170	UDP-N-acetylglucosamine 2-epimerase	1388406 _ 1389470	0.11	0.39
MM_1171	Conserved protein	1389509 _ 1390543	0.13	0.59
MM_1177	Glycosyl transferase	1397659 _ 1396571	-0.02	0.51
MM_1178	Glycosyl transferase	1398824 _ 1397652	-0.01	0.45
MM_1179	Glycosyl transferase	1400079 _ 1398829	-0.46	0.41
MM_1180	Oxidoreductase (hypothetical)	1402277 _ 1400106	-0.18	0.68
MM_1182	Glycosyl transferase	1404766 _ 1403516	-0.05	0.20
MM_1183	Dolichyl-phosphate mannose synthase related protein	1405986 _ 1405015	-0.24	0.51
MM_1184	Dolichyl-phosphate mannose synthase related protein	1407181 _ 1406216	0.09	0.51
MM_1185	Conserved protein	1408105 _ 1408989	-0.19	0.41
MM_1190	Conserved protein	1414602 _ 1416500	-0.64	0.30
MM_1193	dTDP-glucose 4,6-dehydratase	1419207 _ 1418446	-0.17	0.50
MM_1196	Conserved protein	1421632 _ 1422465	-0.03	0.27
MM_1198	Transposase	1425028 _ 1423727	-0.53	0.39
MM_1200	SSU ribosomal protein S17E	1426124 _ 1426315	-0.32	0.41
MM_1201	Dihydrodipicolinate synthase	1426372 _ 1427244	-0.10	0.05
MM_1202	Dihydrodipicolinate reductase	1427244 _ 1428032	-0.14	0.14
MM_1205	Conserved protein	1429957 _ 1430673	0.21	0.62
MM_1206	Conserved protein	1430673 _ 1431287	0.04	0.55
MM_1212	Aspartate carbamoyltransferase, regulatory su	1435217 _ 1434750	0.41	0.34
MM_1213	Aspartate carbamoyltransferase, catalytic su	1436143 _ 1435217	0.23	0.51
MM_1214	Conserved protein	1436723 _ 1436247	0.07	0.41
MM_1215	Hexulose-6-phosphate synthase	1437166 _ 1438392	-0.05	0.59
MM_1216	Conserved protein	1438987 _ 1439454	0.21	0.06
MM_1217	ATP-dependent DNA helicase	1442163 _ 1439476	-0.19	0.31
MM_1218	GMP synthase [glutamine-hydrolyzing]	1443646 _ 1444560	0.01	0.66
MM_1219	Fe-S oxidoreductase	1444746 _ 1445783	-0.37	0.33
MM_1220	Acetylglutamate kinase	1447073 _ 1446198	-0.08	0.44
MM_1221	Chromosomal protein MC1	1447585 _ 1447304	0.03	0.62
MM_1222	Conserved protein	1447990 _ 1448943	-0.08	0.48
MM_1223	Conserved protein	1449203 _ 1449526	-0.07	0.35
MM_1224	Conserved protein	1451107 _ 1449677	-0.13	0.07
MM_1225	F420 hydrogenase/dehydrogenase, beta su	1451901 _ 1453061	0.26	0.22
MM_1226	Glutamyl-tRNA (Gln) amidotransferase, su C	1453229 _ 1453507	-0.17	0.36
MM_1227	Glutamyl-tRNA (Gln) amidotransferase, su A	1453520 _ 1454947	-0.09	0.39
MM_1228	Glutamyl-tRNA (Gln) amidotransferase, su B	1454951 _ 1456435	-0.19	0.45
MM_1229	Nitrilase	1457400 _ 1456480	0.13	0.33
MM_1231	SAM-dependent methyltransferases	1457723 _ 1458253	0.55	0.17
MM_1234	Conserved protein	1461438 _ 1461821	0.39	0.40
MM_1235	5-Methylcytosine-specific restriction enzyme	1461900 _ 1462817	0.57	0.18
MM_1237	Thymidylate synthase	1464649 _ 1465299	0.00	0.47
MM_1238	3-Phosphoshikimate 1-carboxyvinyltransferase	1465406 _ 1466695	0.08	0.34
MM_1239	Methyltransferase	1466831 _ 1467808	0.07	0.46
MM_1240	Methyl-coenzyme M reductase, alpha su	1469869 _ 1468160	0.17	0.41
MM_1241	Methyl-coenzyme M reductase, gamma su	1470635 _ 1469883	-0.01	0.67
MM_1242	Methyl-coenzyme M reductase, operon protein C	1471248 _ 1470631	0.20	0.57
MM_1243	Methyl-coenzyme M reductase, operon protein D	1471767 _ 1471258	0.27	0.47
MM_1244	Methyl-coenzyme M reductase, beta su	1473096 _ 1471795	0.07	0.36

MM_1245	Conserved protein	1473516 _ 1474748	0.21	0.70
MM_1247	Conserved protein	1478847 _ 1478605	-0.28	0.31
MM_1249	Ribulose bisphosphate carboxylase large chain	1481500 _ 1480217	0.14	0.54
MM_1251	Cation transporter	1484467 _ 1483358	0.19	0.27
MM_1252	Conserved protein	1486228 _ 1485119	-0.10	0.46
MM_1253	Cation transporter	1488246 _ 1487137	-0.31	0.49
MM_1254	Putative heat shock protein	1489866 _ 1489372	0.80	0.08
MM_1255	Putative heat shock protein	1490424 _ 1489903	0.26	0.30
MM_1261	Conserved protein	1497298 _ 1496858	0.37	0.46
MM_1262	Xanthine-guanine phosphoribosyltransferase	1498428 _ 1497664	-0.03	0.41
MM_1263	Conserved protein	1498792 _ 1499577	0.07	0.58
MM_1267	Signal recognition particle, su FFH/SRP54	1501802 _ 1503130	0.13	0.41
MM_1270	GMP synthase [glutamine-hydrolyzing]	1505453 _ 1504887	-0.24	0.63
MM_1271	2-Dehydro-3-desoxyphosphoheptanote aldolase	1505915 _ 1506715	-0.52	0.50
MM_1272	3-Dehydroquinate synthase	1506859 _ 1507998	-0.45	0.71
MM_1273	3-Dehydroquinate dehydratase	1508062 _ 1508787	-0.30	0.67
MM_1274	Shikimate 5-dehydrogenase	1508787 _ 1509626	-0.39	0.58
MM_1275	Prephenate dehydrogenase	1509626 _ 1511041	-0.31	0.68
MM_1276	Hypothetical protein	1511119 _ 1512651	-0.30	0.64
MM_1277	Methyltransferase	1513550 _ 1512804	-0.03	0.53
MM_1278	Triosephosphate isomerase	1514554 _ 1513889	0.02	0.46
MM_1279	Orotidine 5'-phosphate decarboxylase family protein	1515783 _ 1514608	-0.01	0.24
MM_1280	Endonuclease III	1516703 _ 1516089	-0.03	0.15
MM_1282	Conserved protein	1518363 _ 1517998	0.29	0.34
MM_1283	Bacterioferritin	1518554 _ 1519021	0.18	0.14
MM_1284	2-Isopropylmalate synthase	1524905 _ 1526452	0.21	0.24
MM_1285	Sec-independent transport protein TatD	1526654 _ 1527424	-0.33	0.45
MM_1287	Fe-S oxidoreductase	1528486 _ 1530363	-0.21	0.36
MM_1288	Conserved protein	1530740 _ 1530462	0.33	0.31
MM_1292	Archaeosine tRNA-ribosyltransferase	1534111 _ 1535979	0.09	0.13
MM_1293	Ribosomal protein S18 alanine acetyltransferase	1536054 _ 1536581	0.58	0.46
MM_1295	DNA primase	1538928 _ 1537327	-0.19	0.21
MM_1296	NADH oxidase	1540365 _ 1541843	-0.24	0.28
MM_1297	RNA 3'-terminal phosphate cyclase	1542052 _ 1543107	-0.12	0.60
MM_1299	Replication factor-A protein	1544636 _ 1546087	0.13	0.62
MM_1300	Transposase	1547852 _ 1546227	0.33	0.75
MM_1302	Conserved protein	1549630 _ 1550421	0.39	0.15
MM_1306	HTH DNA-binding protein	1555090 _ 1554341	-0.15	0.51
MM_1307	Conserved protein	1555307 _ 1556473	0.44	0.15
MM_1310	Hypothetical protein	1559727 _ 1560206	-0.21	0.36
MM_1311	Conserved protein	1561341 _ 1560571	0.01	0.24
MM_1312	Conserved protein	1561934 _ 1561458	-0.17	0.73
MM_1313	UMP/CMP kinase related protein	1562695 _ 1562135	-0.41	0.40
MM_1314	Origin recognition complex su	1564656 _ 1563415	0.16	0.33
MM_1316	Sodium/proline symporter	1566461 _ 1567744	0.17	0.33
MM_1318	Conserved protein	1570823 _ 1571914	0.15	0.08
MM_1319	Pheromone shutdown protein	1572566 _ 1574068	0.09	0.48
MM_1321	Formylmethanofuran--tetrahydromethanopterin formyltransferase	1575910 _ 1575020	0.24	0.41
MM_1322	Chemotaxis protein CheD	1577170 _ 1576694	0.04	0.25
MM_1323	Chemotaxis protein CheC	1578101 _ 1577466	-0.01	0.45
MM_1324	Chemotaxis protein methyltransferase CheR	1579218 _ 1578319	-0.23	0.40
MM_1325	Chemotaxis protein CheA	1581283 _ 1579271	-0.02	0.42
MM_1326	Chemotaxis receptor methylesterase, CheB	1582798 _ 1581698	0.40	0.18
MM_1327	Chemotaxis protein CheY	1583372 _ 1582950	0.33	0.43
MM_1329	Methyl-accepting chemotaxis protein	1585442 _ 1587508	0.58	0.29
MM_1334	Zinc ABC transporter, ATP-binding protein	1591794 _ 1592555	0.29	0.32
MM_1335	Zinc ABC transporter, permease protein	1592607 _ 1593428	0.39	0.47
MM_1336	Hypothetical protein	1594052 _ 1594504	-0.11	0.28
MM_1337	Hypothetical protein	1596833 _ 1595247	0.14	0.56

MM_1339	Pyruvate:ferredoxin oxidoreductase, beta su	1604061 _ 1603174	0.64	0.37
MM_1340	Pyruvate:ferredoxin oxidoreductase, alpha su	1605266 _ 1604061	0.51	0.38
MM_1341	Pyruvate:ferredoxin oxidoreductase, delta su	1605598 _ 1605269	0.59	0.25
MM_1342	Pyruvate:ferredoxin oxidoreductase, gamma su	1606143 _ 1605598	0.38	0.16
MM_1343	NifR3-like protein	1607685 _ 1606717	0.14	0.54
MM_1344	Signal sequence peptidase	1608603 _ 1608049	-0.48	0.13
MM_1345	DNA polymerase II small su	1609877 _ 1611817	-0.27	0.28
MM_1346	Fe-S oxidoreductase	1611885 _ 1612979	-0.09	0.36
MM_1347	Peptide chain release factor	1613824 _ 1615068	-0.23	0.52
MM_1348	Arginyl-tRNA synthetase	1615333 _ 1617039	-0.46	0.34
MM_1349	Conserved protein	1617562 _ 1617969	0.00	0.37
MM_1352	5-Methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	1620523 _ 1621560	0.23	0.30
MM_1353	Conserved protein	1621804 _ 1622835	0.26	0.43
MM_1355	Conserved protein	1624178 _ 1624381	-0.58	0.43
MM_1356	Tram domain protein	1624451 _ 1624654	-0.72	0.24
MM_1357	Tram domain protein	1624722 _ 1624925	-0.46	0.34
MM_1358	Putative methyltransferase	1624990 _ 1625193	-0.23	0.36
MM_1359	Hypothetical protein	1625543 _ 1625968	0.08	0.15
MM_1360	ABC transporter, ATP-binding protein	1626806 _ 1626018	0.27	0.22
MM_1361	ABC transporter, permease protein	1627570 _ 1626785	0.36	0.36
MM_1362	ABC transporter, periplasmic binding protein	1628725 _ 1627739	0.14	0.61
MM_1363	Conserved hypothetical protein	1629101 _ 1629628	0.60	0.41
MM_1364	S-layer protein	1633238 _ 1629747	0.03	0.48
MM_1365	Thiamin-monophosphate kinase	1634425 _ 1633415	-0.18	0.62
MM_1366	Ribonucleoside-triphosphate reductase activating enzyme	1636022 _ 1635234	0.27	0.21
MM_1367	Anaerobic ribonucleoside-triphosphate reductase	1638450 _ 1636069	0.33	0.54
MM_1368	Glutaredoxin-like protein	1638784 _ 1638533	-0.26	0.27
MM_1369	DNA repair protein	1639456 _ 1640199	-0.02	0.36
MM_1370	Myo-inositol-1-phosphate synthase	1641408 _ 1640311	-0.05	0.31
MM_1371	Archaeal protein Translation Initiation Factor 2B su 1 (aIF-2B1)	1641948 _ 1642973	-0.09	0.58
MM_1372	Hypothetical protein	1643373 _ 1643537	-0.06	0.34
MM_1373	Hydrolase of the alpha/beta superfamily	1643541 _ 1644542	-0.22	0.35
MM_1375	Polyphosphate kinase	1647406 _ 1649589	0.22	0.46
MM_1376	Exopolyphosphatase	1649935 _ 1651563	0.24	0.12
MM_1378	Origin recognition complex su	1655076 _ 1656194	0.34	0.46
MM_1379	Thermosome, alpha su	1658018 _ 1656366	-0.09	0.66
MM_1380	Chaperone protein	1658969 _ 1660387	0.11	0.52
MM_1381	Chaperone protein	1660567 _ 1661280	-0.05	0.42
MM_1382	Conserved protein	1662067 _ 1661339	-0.21	0.39
MM_1383	Phenylalanyl-tRNA synthetase, alpha chain	1663733 _ 1662117	0.10	0.12
MM_1384	Superfamily I DNA and RNA helicase	1664729 _ 1664100	0.04	0.78
MM_1385	Conserved protein	1665841 _ 1665563	0.05	0.37
MM_1386	Hypothetical protein	1666078 _ 1666689	0.14	0.17
MM_1387	ATP-dependent RNA helicase	1669413 _ 1666822	0.16	0.36
MM_1388	Glycyl-tRNA synthetase	1671540 _ 1669585	-0.10	0.37
MM_1389	Conserved protein	1672659 _ 1671838	-0.11	0.58
MM_1391	Survival protein	1674930 _ 1675730	0.28	0.51
MM_1393	Molybdenum cofactor biosynthesis protein A	1677174 _ 1676173	0.03	0.64
MM_1394	Cobyrinic acid a,c-diamide synthase	1677516 _ 1678865	-0.16	0.50
MM_1395	Consered protein	1679255 _ 1679716	-0.47	0.63
MM_1396	Conserved protein	1680921 _ 1679812	0.40	0.14
MM_1397	DNA polymerase sliding clamp	1681666 _ 1680932	0.16	0.63
MM_1399	MutT-like protein	1682699 _ 1682292	-0.14	0.37
MM_1401	Hypothetical protein	1683533 _ 1684399	-0.15	0.42
MM_1402	Phosphatidylserine decarboxylase	1684408 _ 1685031	-0.26	0.21
MM_1403	CDP-diacylglycerol--serine O-phosphatidyltransferase	1685037 _ 1685777	0.03	0.42
MM_1404	Conserved protein	1685842 _ 1686195	-0.14	0.31
MM_1405	Histidinol-phosphate aminotransferase	1687431 _ 1686328	-0.09	0.43

MM_1406	Acetylornithine aminotransferase	1688593 _ 1687409	-0.08	0.64
MM_1407	Conserved protein	1690307 _ 1689237	0.38	0.29
MM_1409	Hypothetical protein	1691476 _ 1692588	0.01	0.33
MM_1410	Hypothetical protein	1692725 _ 1693771	-0.28	0.38
MM_1411	Hypothetical protein	1694174 _ 1695307	0.24	0.09
MM_1412	Hypothetical protein	1695362 _ 1695925	0.46	0.16
MM_1413	Conserved protein	1696258 _ 1695959	-0.01	0.34
MM_1415	Conserved protein	1697422 _ 1697793	0.12	0.38
MM_1416	Phosphoribosylformylglycinamidine cyclo-ligase	1699029 _ 1697980	-0.23	0.24
MM_1417	Aspartate kinase	1700440 _ 1699004	-0.08	0.09
MM_1418	3-Phosphonopyruvate decarboxylase	1700806 _ 1702053	0.22	0.33
MM_1419	Small heat shock protein	1702391 _ 1702849	0.27	0.14
MM_1420	Hypothetical protein	1703524 _ 1703108	0.16	0.50
MM_1421	Conserved protein	1703918 _ 1704679	0.20	0.28
MM_1422	Hypothetical protein	1705212 _ 1704892	-0.28	0.56
MM_1423	dCMP deaminase	1705725 _ 1705267	-0.05	0.50
MM_1424	Protein translocase, su SecF	1706127 _ 1707029	-0.06	0.43
MM_1425	Protein translocase, su SecD	1707029 _ 1708726	-0.05	0.34
MM_1426	Replication factor c su	1708793 _ 1709815	0.04	0.61
MM_1428	Conserved protein	1710971 _ 1711543	-0.36	0.11
MM_1430	Transposase	1712988 _ 1712395	-0.24	0.32
MM_1433	Hypothetical protein	1715325 _ 1715092	0.02	0.30
MM_1434	Monomethylamine permease MtmP	1715607 _ 1715341	0.11	0.75
MM_1435	Monomethylamine permease MtmP	1717000 _ 1715597	0.23	0.61
MM_1436	Monomethylamine:corrinoid methyltransferase MtmB1	1718014 _ 1717295	0.34	0.44
MM_1438	Monomethylamine corrinoid protein MtmC1	1719494 _ 1718691	0.04	0.47
MM_1440	Conserved protein	1721763 _ 1723382	0.13	0.40
MM_1441	Inorganic pyrophosphatase	1723726 _ 1724265	-0.04	0.48
MM_1442	Hypothetical protein	1725203 _ 1724412	0.43	0.38
MM_1443	PylC (Pyrrolysine synthesis)	1726356 _ 1725244	0.33	0.44
MM_1445	Lysly-tRNA synthetase (PylS)	1728981 _ 1727620	-0.15	0.26
MM_1446	Hypothetical protein	1729060 _ 1729284	0.34	0.40
MM_1448	Hypothetical protein	1730733 _ 1731263	-0.36	0.52
MM_1449	Conserved protein	1731432 _ 1731821	0.04	0.16
MM_1452	Universal stress protein	1734503 _ 1733535	0.04	0.23
MM_1453	Universal stress protein	1735261 _ 1734812	0.00	0.55
MM_1454	Universal stress protein	1735866 _ 1735381	0.27	0.67
MM_1456	Universal stress protein	1736997 _ 1736506	0.05	0.60
MM_1457	Conserved protein	1738372 _ 1737698	0.11	0.33
MM_1458	Conserved protein	1739710 _ 1738439	0.22	0.42
MM_1459	Hypothetical protein	1739916 _ 1740140	0.16	0.75
MM_1460	Conserved protein	1740462 _ 1740695	-0.54	0.37
MM_1462	Hypothetical protein	1744896 _ 1744354	0.41	0.14
MM_1464	Hypothetical protein	1747797 _ 1747561	0.42	0.53
MM_1468	Hypothetical protein	1751228 _ 1750902	0.19	0.06
MM_1470	Phenylalanyl-tRNA synthetase, alpha chain	1754022 _ 1752538	-0.23	0.26
MM_1471	Tryptophanyl-tRNA synthetase	1755719 _ 1754235	-0.06	0.18
MM_1473	Conserved protein	1756311 _ 1756760	0.33	0.62
MM_1474	Metallo cofactor biosynthesis protein moaA/ nifB /pqqE family	1758053 _ 1756842	0.09	0.54
MM_1475	Probable translation initiation factor 2 beta su	1759113 _ 1758508	-0.07	0.38
MM_1476	LSU ribosomal protein L10E	1759673 _ 1759155	-0.04	0.16
MM_1478	Molybdenum cofactor biosynthesis protein B	1761355 _ 1761891	-0.35	0.22
MM_1480	Conserved protein	1765713 _ 1766648	0.35	0.58
MM_1486	Alanyl-tRNA synthetase	1769820 _ 1772597	0.05	0.51
MM_1491	Putative nucleotidytransferase	1778611 _ 1777271	-0.10	0.72
MM_1493	Conserved protein	1781668 _ 1780601	0.13	0.39
MM_1498	Hypothetical protein	1786476 _ 1785691	-0.08	0.40
MM_1499	Methionine aminopeptidase	1787448 _ 1786561	-0.04	0.49
MM_1500	Xaa-Pro aminopeptidase	1788847 _ 1787669	0.05	0.39

MM_1502	S-adenosylmethionine synthetase	1792087 _ 1790894	-0.14	0.49
MM_1504	Phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	1793554 _ 1794291	0.08	0.30
MM_1505	Imidazoleglycerol-phosphate dehydratase	1794322 _ 1794894	0.01	0.51
MM_1506	FdhD protein	1795701 _ 1794964	-0.26	0.46
MM_1508	Hypothetical protein	1798163 _ 1797240	0.02	0.51
MM_1514	Hypothetical protein	1804253 _ 1803195	0.20	0.16
MM_1515	Transporter	1806251 _ 1804266	-0.19	0.35
MM_1517	Cysteine desulfurase	1809553 _ 1808375	0.29	0.12
MM_1518	Conserved protein	1810831 _ 1810181	-0.31	0.35
MM_1519	Amino-acid acetyltransferase	1811642 _ 1811175	0.21	0.42
MM_1520	Conserved protein	1812498 _ 1811674	0.16	0.24
MM_1521	Phosphoglucomutase/phosphomannomutase	1812789 _ 1814132	0.24	0.34
MM_1522	Putative FeS oxidoreductase	1816217 _ 1815096	-0.18	0.34
MM_1523	Zinc metalloprotease	1817413 _ 1816286	0.14	0.39
MM_1524	Conserved protein	1818046 _ 1817615	0.21	0.65
MM_1525	Conserved protein	1819229 _ 1818036	-0.05	0.30
MM_1526	4-Hydroxybenzoate decarboxylase	1820716 _ 1819451	0.17	0.43
MM_1527	Citrate (si) synthase	1822098 _ 1821034	-0.02	0.42
MM_1528	Aconitate hydratase	1825360 _ 1822535	-0.02	0.53
MM_1529	Hypothetical protein	1826627 _ 1827346	0.51	0.50
MM_1530	Hypothetical protein	1829603 _ 1827885	-0.09	0.56
MM_1531	Putative regulartory protein	1831156 _ 1829639	-0.64	0.34
MM_1534	Hypothetical protein	1832698 _ 1833636	0.53	0.30
MM_1536	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase	1834796 _ 1835404	0.20	0.30
MM_1537	Conserved protein	1835547 _ 1835984	0.20	0.58
MM_1538	Replication factor c su	1836245 _ 1837744	0.13	0.18
MM_1539	Hypothetical protein	1838953 _ 1837826	0.11	0.40
MM_1548	Protoglobin-like protein	1845778 _ 1846584	-0.04	0.44
MM_1549	Sodium/proline symporter	1848581 _ 1846665	-0.32	0.28
MM_1550	putative molybdenum transport protein ModA	1849515 _ 1850096	-0.01	0.35
MM_1551	Hypothetical protein	1851454 _ 1850369	0.23	0.59
MM_1552	Hypothetical protein	1852559 _ 1851423	-0.09	0.44
MM_1553	TRANSPORTER, RND superfamily	1854922 _ 1852559	-0.26	0.51
MM_1554	Transcriptional regulator	1855125 _ 1855901	-0.07	0.70
MM_1555	Putative metal dependent hydrolase	1856032 _ 1856688	-0.34	0.31
MM_1556	Conserved protein	1856756 _ 1857076	0.01	0.30
MM_1557	Signal recognition particle SEC65 su	1857592 _ 1857290	0.00	0.34
MM_1558	Hypothetical protein	1857908 _ 1858162	0.47	0.45
MM_1559	Hypothetical protein	1858409 _ 1859272	0.28	0.64
MM_1563	TUNGSTEN TRANSPORTER, ATP BINDING PROTEIN	1862224 _ 1863282	0.35	0.16
MM_1564	Molybdenum formylmethanofuran dehydrogenase, su	1863884 _ 1864483	0.50	0.21
MM_1565	Molybdenum formylmethanofuran dehydrogenase, su	1864505 _ 1865542	0.49	0.24
MM_1567	Molybdenum formylmethanofuran dehydrogenase, su	1867305 _ 1868168	0.08	0.24
MM_1568	Molybdenum formylmethanofuran dehydrogenase, su	1868140 _ 1868568	0.39	0.37
MM_1569	Molybdenum formylmethanofuran dehydrogenase, su	1868568 _ 1869872	0.37	0.19
MM_1570	Conserved protein	1870586 _ 1870182	0.21	0.45
MM_1571	Conserved protein	1871428 _ 1872174	0.13	0.31
MM_1574	Conserved protein	1876045 _ 1875272	-0.02	0.64
MM_1576	Conserved protein	1878685 _ 1877843	0.62	0.31
MM_1578	Molybdate ABC transporter, ATP-binding protein	1880733 _ 1879675	0.47	0.31
MM_1579	Molybdate ABC transporter, permease protein	1881536 _ 1880733	0.40	0.37
MM_1580	Molybdate-binding protein	1882342 _ 1881527	0.53	0.38
MM_1581	Putative NAD(P)H oxidoreductase	1883169 _ 1882531	-0.05	0.45
MM_1584	NADPH-flavin oxidoreductase	1884783 _ 1884262	0.33	0.30
MM_1585	Iron-sulfur flavoprotein	1885492 _ 1884938	0.55	0.25
MM_1588	Surface layer protein (putative)	1890782 _ 1888830	-0.39	0.48
MM_1589	Surface layer protein (putative)	1894195 _ 1891559	0.07	0.47
MM_1590	Conserved protein	1894880 _ 1895323	-0.25	0.59
MM_1591	Conserved protein	1895432 _ 1896334	-0.11	0.65

MM_1592	Conserved protein	1896354 _ 1897334	0.12	0.35
MM_1593	Probable RNA processing protein	1897334 _ 1898341	0.02	0.43
MM_1595	Conserved protein	1899736 _ 1899194	0.11	0.64
MM_1597	Integral membrane protein	1900894 _ 1901484	0.17	0.67
MM_1598	Conserved protein	1901520 _ 1901924	0.14	0.67
MM_1599	Integral membrane protein	1902064 _ 1902828	-0.21	0.23
MM_1601	Cobalamin biosynthesis protein CobN	1904236 _ 1908582	0.47	0.61
MM_1603	Conserved protein	1914198 _ 1913764	-0.06	0.59
MM_1604	Tungsten formylmethanofuran dehydrogenase su E	1914709 _ 1914194	-0.10	0.42
MM_1605	Conserved protein	1916206 _ 1916991	0.08	0.69
MM_1606	Archaeal protein Translation Initiation Factor 2B su 2aIF-2B2	1917092 _ 1918012	0.26	0.51
MM_1607	Acetyltransferase	1918075 _ 1918536	0.42	0.15
MM_1608	ABC transporter, ATP-binding protein	1919318 _ 1918611	0.00	0.66
MM_1609	ABC transporter, permease protein	1920426 _ 1919314	0.19	0.21
MM_1610	Hypothetical protein	1921669 _ 1920485	-0.01	0.44
MM_1611	Uridylate kinase	1922544 _ 1921846	0.08	0.78
MM_1615	Oxidoreductase, aldo/keto family	1926295 _ 1927488	0.17	0.56
MM_1616	Phosphoribosylaminoimidazole carboxylase	1927818 _ 1928627	0.01	0.16
MM_1617	Conserved protein	1928691 _ 1929878	-0.01	0.55
MM_1618	Aspartate-semialdehyde dehydrogenase	1931124 _ 1930099	0.27	0.55
MM_1619	Ferredoxin	1931403 _ 1931224	0.04	0.76
MM_1620	Conserved protein	1932747 _ 1932442	0.28	0.43
MM_1621	Cobalamin biosynthesis protein CobW	1934009 _ 1932891	0.21	0.59
MM_1625	Conserved protein	1937385 _ 1937059	-0.28	0.58
MM_1626	Conserved protein	1937814 _ 1938806	0.36	0.08
MM_1627	Fructose-bisphosphate aldolase	1940670 _ 1939870	-0.03	0.41
MM_1628	Deoxycytidine triphosphate deaminase	1941563 _ 1941051	0.18	0.15
MM_1629	Conserved protein	1942010 _ 1944070	0.13	0.75
MM_1632	Iron-sulfur flavoprotein	1946470 _ 1947033	0.23	0.44
MM_1633	ABC transporter, ATP-binding protein	1947491 _ 1948699	-0.10	0.34
MM_1634	Transcriptional regulator	1948868 _ 1949287	0.27	0.25
MM_1636	Glycosyltransferase	1951547 _ 1950411	0.19	0.19
MM_1637	Conserved protein	1952644 _ 1952063	0.08	0.91
MM_1638	Glycosyltransferase involved in cell wall biogenesis	1953402 _ 1954319	0.27	0.33
MM_1639	Putative acetyltransferase	1954521 _ 1954970	0.19	0.06
MM_1641	Glycosyl transferase	1956060 _ 1957142	0.11	0.24
MM_1644	Methyltransferase	1961700 _ 1962635	0.17	0.31
MM_1647	Methanol:corrinoid methyltransferase MtaB1	1967450 _ 1966068	0.09	0.65
MM_1648	Methanol corrinoid protein MtaC1	1968267 _ 1967464	0.36	0.42
MM_1649	Conserved protein	1969213 _ 1969980	-0.08	0.29
MM_1650	Conserved protein	1970267 _ 1971055	0.03	0.19
MM_1655	Conserved protein	1978077 _ 1976158	0.19	0.35
MM_1657	Putative serine/threonine protein phosphatase	1980050 _ 1981246	0.20	0.31
MM_1658	methyl-accepting chemotaxis protein	1981521 _ 1983515	0.07	0.33
MM_1661	Type I restriction-modification system restriction su	1985816 _ 1988566	0.44	0.28
MM_1663	Conserved protein	1991146 _ 1989986	0.09	0.16
MM_1664	Conserved protein	1991640 _ 1991146	0.31	0.41
MM_1668	ATP-dependent DNA helicase RecG	1996782 _ 1998230	-0.18	0.21
MM_1670	Conserved protein	2001113 _ 2000106	0.59	0.28
MM_1671	Hypothetical sensory transduction histidine kinase	2001789 _ 2004332	0.40	0.29
MM_1673	Galactoside O-acetyltransferase	2006465 _ 2005767	0.08	0.35
MM_1674	Hypothetical protein	2007169 _ 2006957	0.15	0.04
MM_1675	Conserved protein	2007971 _ 2008645	-0.39	0.19
MM_1676	Conserved protein	2009304 _ 2011109	-0.11	0.61
MM_1678	Conserved protein	2015514 _ 2016287	0.28	0.13
MM_1679	Transposase	2017670 _ 2016429	-0.40	0.10
MM_1681	CbiD protein	2019565 _ 2018549	0.20	0.53
MM_1682	DNA mismatch repair protein	2022310 _ 2020244	0.11	0.67
MM_1683	DNA mismatch repair protein	2025232 _ 2022533	0.05	0.33

MM_1684	O-acetyl transferase	2027136 _ 2025967	0.17	0.45
MM_1691	Trimethylamine permease	2034036 _ 2035094	-0.32	0.16
MM_1692	Conserved protein	2035099 _ 2035389	0.54	0.41
MM_1694	Dimethylamine:corrinoid methyltransferase MtbB1	2036708 _ 2037016	0.40	0.40
MM_1695	Conserved protein	2037622 _ 2038260	0.15	0.35
MM_1696	Lysyl-tRNA synthetase, class I	2038955 _ 2040574	-0.12	0.41
MM_1697	LysM	2040610 _ 2041311	0.32	0.54
MM_1698	Conserved protein	2041542 _ 2041345	0.58	0.55
MM_1699	Conserved protein	2042236 _ 2041754	-0.42	0.35
MM_1701	Putative nucleoside-diphosphate-sugar epimerase	2045020 _ 2045901	-0.19	0.58
MM_1703	Conserved protein	2047509 _ 2047225	-0.18	0.61
MM_1704	Histidine biosynthesis protein	2048501 _ 2047683	0.11	0.43
MM_1706	Protein-L-isoaspartate O-methyltransferase	2050039 _ 2049311	0.06	0.64
MM_1707	Conserved protein	2050616 _ 2049984	0.12	0.49
MM_1708	Conserved protein	2051239 _ 2050838	0.23	0.29
MM_1709	Conserved protein	2051522 _ 2052196	0.14	0.52
MM_1711	Cobalt-zinc-cadmium resistance protein	2053311 _ 2054228	0.37	0.45
MM_1712	Chorismate synthase	2054237 _ 2055331	0.20	0.55
MM_1713	Conserved protein	2055906 _ 2058179	0.05	0.72
MM_1715	Conserved protein	2059333 _ 2059740	0.45	0.65
MM_1716	Transcriptional regulator, ArsR family	2060597 _ 2059785	0.64	0.17
MM_1717	Conserved protein	2061719 _ 2062894	-0.41	0.24
MM_1722	Hypothetical protein	2066302 _ 2067336	-0.47	0.55
MM_1723	Conserved protein	2067511 _ 2068155	-0.01	0.08
MM_1724	Hypothetical protein	2068242 _ 2068667	0.21	0.25
MM_1726	Heme biosynthesis protein	2069622 _ 2070686	0.14	0.69
MM_1728	Heme biosynthesis protein	2071217 _ 2071690	-0.05	0.34
MM_1731	Conserved protein	2073444 _ 2073851	-0.10	0.09
MM_1732	Transcriptional regulator, ArsR family	2074709 _ 2073897	0.53	0.42
MM_1733	Metalloproteinase	2075197 _ 2075682	-0.16	0.36
MM_1734	Conserved protein	2075699 _ 2076316	-0.50	0.23
MM_1735	Transposase (N-terminal domain)	2076486 _ 2076791	-0.13	0.32
MM_1737	Heme biosynthesis protein	2078638 _ 2079684	-0.05	0.36
MM_1738	Heme biosynthesis protein	2079699 _ 2080226	-0.11	0.51
MM_1739	Heme biosynthesis protein	2080233 _ 2080706	0.19	0.37
MM_1741	Glutamyl-tRNA reductase	2081770 _ 2083113	-0.03	0.31
MM_1742	Delta-aminolevulinic acid dehydratase	2083290 _ 2084261	0.18	0.66
MM_1743	Glutamate-1-semialdehyde 2,1-aminomutase	2084644 _ 2085915	-0.06	0.47
MM_1744	Porphobilinogen deaminase	2085981 _ 2086928	-0.18	0.23
MM_1745	Dihydroorotate dehydrogenase	2086931 _ 2087872	-0.06	0.29
MM_1746	Dihydroorotate dehydrogenase electron transfer su	2087875 _ 2088651	-0.09	0.21
MM_1747	2-Hydroxy-2,4-diene-1,7-dioate isomerase	2088732 _ 2089463	0.06	0.48
MM_1748	Conserved protein	2089466 _ 2089768	0.21	0.19
MM_1749	Glutamyl-tRNA synthetase	2090005 _ 2091717	0.17	0.67
MM_1752	Conserved protein	2094693 _ 2095559	-0.11	0.13
MM_1753	D-3-phosphoglycerate dehydrogenase	2095657 _ 2097276	-0.03	0.23
MM_1754	Conserved protein	2097514 _ 2098029	-0.13	0.20
MM_1755	LSU ribosomal protein L18E	2098200 _ 2098577	-0.09	0.34
MM_1756	LSU ribosomal protein L13P	2098525 _ 2099010	0.02	0.20
MM_1757	SSU ribosomal protein S9P	2099027 _ 2099428	-0.26	0.21
MM_1758	DNA-directed RNA polymerase su N	2099440 _ 2099625	-0.17	0.28
MM_1761	Conserved protein	2100878 _ 2101678	0.06	0.53
MM_1762	Mevalonate kinase	2101873 _ 2102775	0.02	0.33
MM_1763	Archaeal kinase	2102912 _ 2103691	0.02	0.30
MM_1764	Isopentenyl-diphosphate delta-isomerase	2103698 _ 2104792	-0.39	0.47
MM_1765	Hypothetical protein	2105598 _ 2105825	0.09	0.28
MM_1766	Zn-dependent hydrolase	2106303 _ 2107643	0.10	0.79
MM_1767	Geranyltranstransferase	2107673 _ 2108644	0.13	0.24
MM_1768	Hypothetical protein	2110126 _ 2109335	-0.26	0.57
MM_1770	Pyruvate, phosphate dikinase	2114480 _ 2111829	0.44	0.15

MM_1771	Hypothetical protein	2115291 _ 2115557	0.06	0.25
MM_1772	Transcription initiation factor IIB	2115751 _ 2116761	0.08	0.68
MM_1773	Conserved protein	2117165 _ 2117815	0.08	0.57
MM_1775	Methyltransferase	2118302 _ 2119309	-0.09	0.33
MM_1776	Thiol-specific antioxidant protein	2120249 _ 2119788	0.11	0.51
MM_1779	Hypothetical protein	2124622 _ 2123945	0.11	0.69
MM_1780	Hypothetical protein	2125319 _ 2124657	0.05	0.47
MM_1782	Sodium/glutamate symport carrier protein	2128309 _ 2129730	0.22	0.66
MM_1784	Hypothetical protein	2131138 _ 2132697	0.21	0.63
MM_1785	Hypothetical protein	2132766 _ 2133956	-0.22	0.44
MM_1787	Transposase	2134645 _ 2135775	-0.08	0.40
MM_1788	HTH DNA-binding protein	2135879 _ 2136733	0.05	0.49
MM_1789	Hypothetical protein	2137915 _ 2137001	0.03	0.49
MM_1790	Conserved protein	2138403 _ 2139242	0.39	0.48
MM_1791	Conserved protein	2139329 _ 2139937	0.34	0.36
MM_1795	Iron-sulfur cluster-binding protein	2145190 _ 2144054	0.40	0.32
MM_1797	10 kDa chaperonin	2145972 _ 2146247	0.06	0.35
MM_1798	GroEL protein	2146398 _ 2148005	-0.09	0.46
MM_1799	Conserved protein	2148274 _ 2149098	0.07	0.71
MM_1800	Transporter	2149409 _ 2150095	-0.15	0.21
MM_1801	Conserved protein	2150204 _ 2150842	0.41	0.20
MM_1802	Aspartate aminotransferase	2151057 _ 2152238	0.04	0.52
MM_1804	Rubrerythrin	2154415 _ 2153894	0.31	0.77
MM_1805	Conserved protein	2156335 _ 2154935	-0.10	0.52
MM_1807	Protein translation initiation factor 2 su alpha IF-2a	2158195 _ 2157383	-0.07	0.53
MM_1808	SSU ribosomal protein S27E	2158447 _ 2158262	0.04	0.08
MM_1809	LSU ribosomal protein L44E	2158732 _ 2158457	0.15	0.30
MM_1810	Conserved protein	2159845 _ 2159123	-0.22	0.39
MM_1811	DNA primase small su	2161055 _ 2159814	0.21	0.68
MM_1813	Conserved protein	2162571 _ 2163791	-0.07	0.31
MM_1815	Cell division protein	2164907 _ 2165956	0.06	0.49
MM_1816	Conserved protein	2166356 _ 2167630	0.14	0.30
MM_1817	Conserved protein	2168977 _ 2168261	0.33	0.13
MM_1818	Conserved protein	2170013 _ 2169141	0.01	0.45
MM_1821	Replication factor c su	2172935 _ 2173948	0.09	0.70
MM_1823	Putative small multi-drug export protein	2174204 _ 2174713	-0.11	0.23
MM_1824	Ferredoxin	2174919 _ 2175188	0.29	0.61
MM_1825	DNA binding protein	2175479 _ 2175270	0.11	0.37
MM_1827	Pyruvate carboxylase (biotin-containing) su B	2177306 _ 2179024	0.48	0.77
MM_1828	Pyruvate carboxylase, su A	2179038 _ 2180528	0.56	0.65
MM_1829	Biotin-[acetyl-CoA-carboxylase] synthetase/biotin operon repressor	2180649 _ 2181623	0.15	0.17
MM_1830	Conserved protein	2181652 _ 2182227	0.01	0.23
MM_1832	Transposase	2183165 _ 2184295	0.03	0.54
MM_1833	Phosphoserine phosphatase	2185275 _ 2184316	-0.26	0.63
MM_1834	Conserved protein	2185929 _ 2185309	0.13	0.34
MM_1835	Conserved protein	2186350 _ 2186063	-0.36	0.37
MM_1836	Cell division control protein (MCM family)	2188602 _ 2186500	-0.23	0.45
MM_1837	RNA methylase	2189508 _ 2188732	0.03	0.35
MM_1839	Conserved protein	2190887 _ 2191678	0.33	0.51
MM_1840	Conserved protein	2191645 _ 2192718	0.03	0.33
MM_1842	Sulfite reductase, assimilatory-type	2193716 _ 2194387	-0.02	0.38
MM_1843	Heterodisulfide reductase, su HdrE	2195032 _ 2195808	0.17	0.48
MM_1844	Heterodisulfide reductase, su HdrD	2195780 _ 2197039	0.18	0.50
MM_1845	Conserved protein	2197153 _ 2197785	-0.11	0.48
MM_1846	Conserved protein	2197781 _ 2198401	-0.22	0.26
MM_1847	Hypothetical protein	2198571 _ 2199566	-0.09	0.21
MM_1849	Geranylgeranyl reductase	2199907 _ 2201112	0.33	0.46
MM_1850	RNase P	2201306 _ 2201812	0.48	0.71
MM_1851	Hypothetical protein	2201994 _ 2203109	-0.21	0.22

MM_1852	Conserved protein	2203099 _ 2204151	-0.13	0.42
MM_1853	Conserved protein	2204151 _ 2204789	-0.04	0.51
MM_1854	Archaeal flavoprotein	2204860 _ 2205576	0.16	0.38
MM_1856	Dipeptide/ oligopeptide ABC transporter ATP-binding protein	2207459 _ 2206509	-0.06	0.54
MM_1857	Dipeptide/ oligopeptide ABC transporter, permease	2208424 _ 2207459	0.13	0.55
MM_1858	Dipeptide/ oligopeptide ABC transporter, permease	2209368 _ 2208424	0.13	0.53
MM_1862	Transporter	2215295 _ 2214090	-0.37	0.31
MM_1863	Transcriptional regulator, MarR family	2215782 _ 2215315	-0.34	0.28
MM_1865	Conserved protein	2216691 _ 2217053	0.44	0.26
MM_1869	Conserved protein	2219327 _ 2220673	0.24	0.57
MM_1870	Conserved protein	2220642 _ 2222822	0.13	0.73
MM_1871	Vanillate decarboxylase protein	2225167 _ 2224586	0.13	0.20
MM_1872	Conserved protein	2225547 _ 2225170	-0.45	0.43
MM_1873	Phosphohydrolase	2226770 _ 2225556	-0.05	0.71
MM_1874	Methyltransferase	2226908 _ 2227816	0.10	0.21
MM_1875	Conserved protein	2228317 _ 2230440	-0.24	0.69
MM_1876	Purine phosphoribosyltransferase	2230892 _ 2231458	0.14	0.31
MM_1877	Diphthamide synthase su	2231427 _ 2232446	-0.05	0.53
MM_1878	Methyltransferase	2232533 _ 2233123	-0.35	0.20
MM_1879	putative RNA-binding protein	2233429 _ 2234376	0.08	0.45
MM_1880	DNA-directed RNA polymerase su L	2234475 _ 2234750	-0.12	0.80
MM_1881	Conserved protein	2235058 _ 2236215	-0.11	0.41
MM_1883	Conserved protein	2236960 _ 2237511	-0.15	0.38
MM_1885	Diaminopimelate decarboxylase	2239794 _ 2238535	-0.18	0.45
MM_1888	Transposase	2243845 _ 2242220	0.26	0.72
MM_1892	Arylsulfatase	2248903 _ 2247368	0.28	0.22
MM_1895	ATP-dependent DNA ligase	2254613 _ 2252877	-0.07	0.74
MM_1896	Phenylacetic acid degradation protein	2255508 _ 2255050	0.03	0.49
MM_1897	Hypothetical protein	2256297 _ 2258156	0.21	0.55
MM_1899	Hypothetical protein	2259651 _ 2259487	-0.10	0.30
MM_1900	Hypothetical protein	2260303 _ 2259755	-0.19	0.28
MM_1902	Hypothetical protein	2262094 _ 2261606	-0.19	0.22
MM_1903	Transcriptional regulator, MarR family	2262781 _ 2262308	0.17	0.20
MM_1904	Hypothetical membrane spanning protein	2263194 _ 2263634	-0.08	0.22
MM_1905	Hypothetical membrane spanning protein	2264373 _ 2263906	-0.07	0.53
MM_1907	Isochorismatase	2265722 _ 2265183	0.18	0.13
MM_1909	Glutathione-regulated potassium-efflux system protein	2266870 _ 2268882	-0.05	0.30
MM_1910	Maf protein	2268889 _ 2269569	0.02	0.48
MM_1911	Cysteinyl-tRNA synthetase	2269793 _ 2271211	0.22	0.53
MM_1913	ATP-dependent protease La	2271899 _ 2272813	0.43	0.39
MM_1914	Hypothetical protein	2273206 _ 2274060	-0.16	0.46
MM_1916	Lysyl-tRNA synthetase, class II	2276788 _ 2278320	-0.43	0.30
MM_1917	ATP-dependent RNA helicase	2278643 _ 2281534	-0.07	0.68
MM_1918	Hypothetical protein	2284464 _ 2281768	-0.24	0.50
MM_1920	Nucleotidyltransferase	2285335 _ 2285622	-0.15	0.31
MM_1922	Hypothetical protein	2286575 _ 2286072	0.12	0.42
MM_1923	Hypothetical protein	2287321 _ 2287530	0.01	0.22
MM_1925	hypothetical protein	2288402 _ 2288013	0.35	0.03
MM_1931	Sensory Transduction protein Kinase	2298295 _ 2300016	-0.13	0.39
MM_1933	Integral membrane protein	2302174 _ 2301263	0.18	0.29
MM_1934	Hypothetical protein	2302331 _ 2303047	-0.07	0.43
MM_1935	Acetyltransferase	2304011 _ 2303154	-0.02	0.42
MM_1936	Conserved protein	2305212 _ 2304091	-0.07	0.50
MM_1937	Hypothetical protein	2305807 _ 2305478	-0.34	0.25
MM_1939	Glutamine-binding protein	2307683 _ 2308534	-0.03	0.54
MM_1942	Polyprenyltransferase	2310451 _ 2311341	-0.21	0.55
MM_1943	Conserved protein	2311637 _ 2312389	-0.33	0.13
MM_1944	Transporter	2312459 _ 2313436	-0.35	0.24
MM_1947	Integral membrane protein	2316132 _ 2315191	-0.19	0.23

MM_1949	Methyltransferase	2318901 _ 2318104	0.05	0.49
MM_1950	Catalase	2319473 _ 2321602	0.12	0.41
MM_1955	Cysteine desulfurase NifS	2326123 _ 2324972	-0.19	0.62
MM_1957	Hypothetical transcriptional regulatory protein	2327095 _ 2326871	0.36	0.37
MM_1958	Hit-like protein, involved in cell-cycle regulation	2327708 _ 2327244	0.27	0.62
MM_1959	Hypothetical protein	2327857 _ 2328165	-0.01	0.40
MM_1960	Conserved protein	2328477 _ 2328788	0.10	0.65
MM_1961	Hypothetical protein	2331071 _ 2328912	0.09	0.59
MM_1962	Conserved protein	2332773 _ 2331604	0.12	0.41
MM_1963	Tyrosyl-tRNA synthetase	2333900 _ 2332950	-0.11	0.48
MM_1964	hypothetical protein	2334670 _ 2333942	0.17	0.12
MM_1965	Hypothetical protein	2334948 _ 2334694	-0.06	0.20
MM_1966	Malate dehydrogenase	2336584 _ 2335664	-0.03	0.31
MM_1967	Hypothetical protein	2337049 _ 2337795	0.11	0.29
MM_1968	Cupin-type phosphoglucose isomerases	2338029 _ 2338766	0.09	0.53
MM_1969	Hypothetical protein	2338990 _ 2339745	0.43	0.25
MM_1970	Single-stranded-DNA-specific exonuclease RecJ	2341286 _ 2339889	0.08	0.55
MM_1971	Conserved protein	2341821 _ 2341282	0.12	0.37
MM_1973	Conserved protein	2342631 _ 2342239	-0.04	0.34
MM_1974	Histone acetyltransferase (ELP3 faminly)	2345770 _ 2344106	-0.15	0.47
MM_1975	Conserved protein	2346816 _ 2346229	0.40	0.24
MM_1976	Conserved protein	2347826 _ 2349832	0.08	0.49
MM_1977	Conserved protein	2350005 _ 2350580	-0.31	0.31
MM_1978	Tungsten formylmethanofuran dehydrogenase su C	2351645 _ 2350890	0.31	0.28
MM_1979	Tungsten formylmethanofuran dehydrogenase su A	2353364 _ 2351649	-0.58	0.29
MM_1980	Tungsten formylmethanofuran dehydrogenase su B	2354670 _ 2353369	-0.55	0.29
MM_1981	Tungsten formylmethanofuran dehydrogenase su D	2355129 _ 2354749	-0.40	0.21
MM_1982	Alkyl sulfatase	2356529 _ 2358250	-0.24	0.48
MM_1983	Conserved protein	2358769 _ 2363592	-0.17	0.29
MM_1984	Hypothetical protein	2364161 _ 2364604	-0.14	0.30
MM_1985	Hypothetical protein	2364814 _ 2365278	0.28	0.44
MM_1986	Regulatory protein (putative)	2365683 _ 2366633	-0.09	0.31
MM_1990	Hypothetical protein	2369536 _ 2368613	0.28	0.40
MM_1995	Cobalt ABC transporter, permease protein CbiQ	2373359 _ 2372469	-0.03	0.24
MM_1997	Cobalt transport permease CbiM analog	2376840 _ 2375269	0.06	0.42
MM_1998	Cobalamin biosynthesis protein CobN	2380766 _ 2376855	-0.32	0.60
MM_1999	Hypothetical protein	2382494 _ 2380779	-0.15	0.69
MM_2000	Hypothetical protein	2384019 _ 2382502	0.52	0.49
MM_2002	Conserved protein	2385083 _ 2386774	-0.38	0.50
MM_2003	Magnesium-chelatase su	2386798 _ 2388873	0.12	0.58
MM_2004	DNA polymerase delta catalytic su	2388985 _ 2391783	0.10	0.38
MM_2005	Phosphate-binding protein	2392304 _ 2393209	-0.21	0.48
MM_2006	Phosphate transporter, permease protein	2393259 _ 2394146	-0.01	0.55
MM_2007	Phosphate transporter, permease protein	2394152 _ 2394649	-0.22	0.30
MM_2009	Phosphate transporter, ATP-binding protein	2395150 _ 2395923	-0.17	0.39
MM_2010	Phosphate transport system protein	2395923 _ 2396579	0.12	0.16
MM_2011	Dihydroorotase	2396738 _ 2398105	-0.07	0.55
MM_2012	Putative RNA-binding protein	2398729 _ 2398184	-0.10	0.49
MM_2013	Serine/threonine protein kinase	2399514 _ 2398729	-0.20	0.19
MM_2014	Translation initiation factor 1A (EIF-1A)	2399792 _ 2399520	-0.23	0.66
MM_2017	Conserved protein	2408461 _ 2407325	-0.11	0.35
MM_2018	Conserved protein	2408623 _ 2410599	0.34	0.41
MM_2019	Phosphoribosyl-AMP cyclohydrolase	2410777 _ 2411136	-0.40	0.28
MM_2020	TWITCHING MOBILITY (PilT) related protein	2411349 _ 2413265	0.29	0.44
MM_2022	TRANSPORTER, LysE family	2414777 _ 2415391	-0.07	0.78
MM_2025	Hypothetical protein	2418771 _ 2419163	0.35	0.82
MM_2026	Hypothetical protein	2419617 _ 2420201	0.07	0.62
MM_2027	Hypothetical protein	2420648 _ 2420226	0.25	0.42
MM_2028	Hypothetical protein	2424741 _ 2421739	0.00	0.17
MM_2029	Conserved protein	2425620 _ 2425832	0.30	0.37

MM_2030	Amidotransferase hisH	2425954 _ 2426574	0.36	0.39
MM_2031	Hydrogenase expression/formation protein	2426798 _ 2428138	0.09	0.29
MM_2032	Nodulation protein	2429501 _ 2428200	-0.16	0.43
MM_2033	Stomatin-like protein	2430344 _ 2429565	0.09	0.41
MM_2034	Conserved protein	2430902 _ 2433031	-0.08	0.53
MM_2035	Orotate phosphoribosyltransferase	2433845 _ 2433234	0.18	0.59
MM_2036	Conserved protein	2434578 _ 2434012	-0.45	0.55
MM_2039	SSU ribosomal protein S8E	2436779 _ 2437153	-0.16	0.42
MM_2041	Aspartate aminotransferase	2437800 _ 2438978	-0.11	0.29
MM_2042	Archaeal transcriptional regulator	2439532 _ 2439125	-0.17	0.44
MM_2043	Hypothetical protein	2439798 _ 2440628	-0.15	0.44
MM_2044	Conserved protein	2441356 _ 2440811	-0.14	0.47
MM_2046	Trimethylamine permease MttP2	2443410 _ 2442364	-0.14	0.36
MM_2048	Trimethylamine:corrinoid methyltransferase MttB2	2444608 _ 2444171	-0.01	0.15
MM_2053	Conserved protein	2449207 _ 2448848	0.03	0.34
MM_2054	ABC transporter, ATP-binding protein	2450288 _ 2449329	0.05	0.46
MM_2055	ABC transporter, ATP-binding protein	2450999 _ 2450295	-0.28	0.54
MM_2056	AdoCbi-P nucleotidyltransferase CobY	2451656 _ 2451048	-0.30	0.60
MM_2057	Cobalamin [5'-phosphate] synthase CobS	2452483 _ 2451644	-0.52	0.27
MM_2058	Hypothetical protein	2453043 _ 2452504	-0.50	0.37
MM_2059	Cobalamin biosynthesis protein CbiB	2454372 _ 2453389	-0.05	0.74
MM_2061	Histidyl-tRNA synthetase	2456360 _ 2457592	-0.04	0.63
MM_2063	Conserved protein	2458408 _ 2459034	-0.03	0.45
MM_2065	Conserved protein	2459679 _ 2460917	-0.53	0.29
MM_2066	SSU ribosomal protein S15P	2461208 _ 2461663	-0.24	0.41
MM_2067	Ferric enterobactin ABC transporter, ATP-binding protein (FepC)	2462691 _ 2461885	0.11	0.53
MM_2069	Ferric enterobactin ABC transporter, solute binding protein (FepB)	2464991 _ 2463780	-0.06	0.63
MM_2070	Nicotinate-nucleotide pyrophosphorylase	2466412 _ 2465582	0.08	0.55
MM_2071	Conserved protein	2469413 _ 2466807	-0.37	0.41
MM_2072	Putative dinucleotide-utilizing enzyme	2470547 _ 2471197	0.03	0.20
MM_2073	Quinolinate synthetase A	2471811 _ 2472722	-0.44	0.50
MM_2074	Conserved protein	2473772 _ 2473131	0.01	0.43
MM_2077	Conserved protein	2478291 _ 2477551	0.20	0.48
MM_2079	Thioredoxin	2479613 _ 2480002	0.54	0.29
MM_2080	Galactoside O-acetyltransferase	2480911 _ 2480111	0.02	0.32
MM_2081	ABC transporter, ATP-binding protein	2481719 _ 2481069	-0.25	0.34
MM_2082	Deoxyhypusine synthase	2482032 _ 2482982	-0.09	0.48
MM_2083	Orotidine 5'-phosphate decarboxylase	2482990 _ 2483649	-0.19	0.42
MM_2092	CODH nickel-insertion accessory protein (iron-sulfur protein)	2496904 _ 2497764	0.08	0.55
MM_2093	Indolepyruvate oxidoreductase, su	2498379 _ 2500178	-0.02	0.35
MM_2094	Indolepyruvate oxidoreductase, su	2500178 _ 2500777	0.14	0.26
MM_2095	Hypothetical protein	2501410 _ 2500874	0.27	0.48
MM_2096	UDP-N-acetylglucosamine-1-phosphate transferase	2501719 _ 2502693	-0.18	0.47
MM_2097	Hypothetical protein	2503362 _ 2502802	-0.12	0.25
MM_2098	Conserved protein	2504564 _ 2503557	0.35	0.23
MM_2099	Hypothetical protein	2509110 _ 2507956	-0.10	0.48
MM_2100	Conserved protein	2511058 _ 2510273	-0.32	0.39
MM_2101	Heteropolysaccharide repeat unit export protein	2512609 _ 2511152	-0.25	0.44
MM_2103	Putative Methyltransferase	2514510 _ 2513779	0.42	0.27
MM_2105	Putative glycosyltransferase	2517307 _ 2516180	-0.41	0.20
MM_2106	Putative glycosyltransferase	2518451 _ 2517339	-0.19	0.71
MM_2108	Conserved protein	2521143 _ 2519611	-0.22	0.43
MM_2109	Glycosyl transferase	2522077 _ 2521187	-0.04	0.44
MM_2111	Galactoside O-acetyltransferase	2523531 _ 2523163	-0.02	0.03
MM_2113	Hypothetical protein	2525518 _ 2524697	-0.03	0.49
MM_2114	Hypothetical protein	2525927 _ 2526769	0.07	0.21
MM_2115	Protease HTPX homolog	2528087 _ 2526900	0.12	0.47
MM_2116	Hypothetical protein	2528368 _ 2528613	0.20	0.33

MM_2117	Hypothetical protein	2528662 _ 2531082	0.24	0.65
MM_2118	Hypothetical protein	2531905 _ 2532390	-0.23	0.60
MM_2121	Hypothetical protein	2535233 _ 2535634	-0.05	0.24
MM_2122	Putative serine/threonine protein kinase	2536074 _ 2537261	-0.41	0.41
MM_2124	LSU ribosomal protein L3P	2538501 _ 2539511	-0.20	0.60
MM_2125	LSU ribosomal protein L4	2539521 _ 2540279	-0.24	0.40
MM_2126	LSU ribosomal protein L23P	2540279 _ 2540524	-0.32	0.31
MM_2127	LSU ribosomal protein L2P	2540538 _ 2541251	-0.13	0.33
MM_2128	SSU ribosomal protein S19P	2541270 _ 2541677	-0.24	0.29
MM_2129	LSU ribosomal protein L22P	2541692 _ 2542144	-0.37	0.16
MM_2130	SSU ribosomal protein S3P	2542148 _ 2543068	-0.17	0.23
MM_2131	LSU ribosomal protein L29P	2543071 _ 2543271	-0.27	0.07
MM_2132	RNaseP suP29	2543264 _ 2543593	-0.24	0.11
MM_2133	SSU ribosomal protein S17P	2543460 _ 2543927	-0.18	0.20
MM_2134	LSU ribosomal protein L14P	2543870 _ 2544322	-0.45	0.16
MM_2135	LSU ribosomal protein L24P	2544318 _ 2544683	-0.41	0.17
MM_2136	SSU ribosomal protein S4E	2544700 _ 2545404	-0.39	0.33
MM_2137	LSU ribosomal protein L5P	2545400 _ 2545906	-0.23	0.32
MM_2138	SSU ribosomal protein S14P	2545910 _ 2546059	-0.51	0.11
MM_2139	SSU ribosomal protein S8P	2546073 _ 2546462	-0.35	0.12
MM_2140	LSU ribosomal protein L6P	2546480 _ 2547007	-0.49	0.27
MM_2141	LSU ribosomal protein L32E	2547021 _ 2547452	-0.48	0.29
MM_2142	LSU ribosomal protein L19E	2547448 _ 2547906	-0.25	0.14
MM_2143	LSU ribosomal protein L18P	2547881 _ 2548447	-0.36	0.31
MM_2144	SSU ribosomal protein S5P	2548460 _ 2549086	-0.18	0.11
MM_2145	LSU ribosomal protein L30P	2549091 _ 2549549	-0.45	0.38
MM_2146	LSU ribosomal protein L15P	2549563 _ 2549982	-0.19	0.22
MM_2147	Protein translocase, su SecY	2550126 _ 2551598	-0.32	0.10
MM_2148	Adenylate kinase	2551694 _ 2552338	-0.28	0.14
MM_2149	Phosphoribosylaminoimidazole carboxylase	2552548 _ 2553054	-0.47	0.34
MM_2150	Conserved protein	2553465 _ 2553740	-0.08	0.35
MM_2152	Hypothetical protein	2555012 _ 2555629	-0.25	0.20
MM_2154	tRNA pseudouridine synthase	2556353 _ 2557366	-0.01	0.47
MM_2155	SSU ribosomal protein S13P	2557860 _ 2558345	0.07	0.40
MM_2156	SSU ribosomal protein S4P	2558370 _ 2559026	-0.07	0.41
MM_2158	DNA-directed RNA polymerase, su D	2559485 _ 2560282	-0.15	0.32
MM_2159	Hypothetical protein	2562898 _ 2561441	-0.21	0.39
MM_2160	Hypothetical protein	2563737 _ 2564165	0.18	0.40
MM_2163	Hydrogenase expression/formation protein	2566961 _ 2566278	0.00	0.30
MM_2171	F420-nonreducing hydrogenase II, cytochrome b su (VhtC)	2574334 _ 2575143	-0.04	0.50
MM_2172	Hydrogenase expression/formation protein	2575265 _ 2575747	-0.05	0.20
MM_2174	Conserved protein	2577509 _ 2576583	-0.19	0.36
MM_2178	Two component system histidine kinase	2584762 _ 2582324	-0.55	0.20
MM_2180	GMP synthase [glutamine-hydrolyzing]	2586450 _ 2585731	-0.34	0.30
MM_2181	Fructose-1,6-bisphosphatase	2586726 _ 2587811	-0.25	0.53
MM_2182	Hypothetical protein	2588166 _ 2589470	-0.11	0.47
MM_2183	Hypothetical protein	2589921 _ 2589667	-0.26	0.18
MM_2186	Conserved protein	2594906 _ 2595511	-0.22	0.32
MM_2188	Conserved protein	2596377 _ 2596973	-0.08	0.31
MM_2189	Rubrerythrin	2597683 _ 2597138	0.18	0.33
MM_2190	Hypothetical protein	2599098 _ 2598271	0.15	0.42
MM_2192	Transposase	2600181 _ 2601662	0.35	0.32
MM_2193	DNA repair protein (MRE11/RAD32 family)	2601848 _ 2603698	-0.30	0.26
MM_2194	DNA repair protein RAD50	2603698 _ 2606907	-0.05	0.21
MM_2195	F420-dependent glucose-6-phosphate dehydrogenase	2608115 _ 2607120	-0.30	0.21
MM_2196	Conserved protein	2609350 _ 2608538	0.31	0.53
MM_2197	Conserved protein	2610504 _ 2609710	0.34	0.37
MM_2199	Type I restriction-modification system specificity su	2612756 _ 2613907	-0.30	0.30
MM_2200	Hypothetical protein	2614093 _ 2614524	0.39	0.06

MM_2201	Hypothetical protein	2614484 _ 2615440	0.05	0.29
MM_2202	Hypothetical protein	2615446 _ 2615772	-0.29	0.40
MM_2203	Hypothetical protein	2615782 _ 2616813	-0.28	0.33
MM_2205	Ribose-phosphate pyrophosphokinase	2621195 _ 2620311	0.12	0.64
MM_2208	Putative sugar kinase	2623825 _ 2622326	-0.08	0.31
MM_2209	Conserved protein	2624641 _ 2626104	0.07	0.34
MM_2210	Oligosaccharyl transferase	2628924 _ 2626429	-0.19	0.44
MM_2211	Hypothetical protein	2630338 _ 2632044	-0.05	0.60
MM_2213	Putative glycosyltransferase	2638184 _ 2634045	-0.32	0.38
MM_2215	polysaccharide ABC transporter permease	2639707_2640468	-0.31	0.32
MM_2216	Glucose-1-phosphate thymidylyltransferase	2641228 _ 2640485	-0.26	0.47
MM_2217	Conserved protein	2642360 _ 2643301	0.25	0.56
MM_2218	Transposase	2644478 _ 2643375	-0.24	0.22
MM_2219	Hypothetical protein	2645525 _ 2644941	-0.23	0.41
MM_2222	Galactoside O-acetyltransferase	2649341 _ 2648172	-0.15	0.32
MM_2223	Dolichyl-phosphate glucose synthetase	2650496 _ 2649354	-0.02	0.25
MM_2224	Trp repressor binding protein	2650713 _ 2651339	-0.33	0.67
MM_2225	Glucoamylase	2652173 _ 2653708	0.04	0.21
MM_2226	Glucoamylase	2653471 _ 2654196	0.11	0.21
MM_2228	Amidohydrolase (putative)	2656114 _ 2654831	0.47	0.58
MM_2230	Transposase	2658095 _ 2657475	0.05	0.34
MM_2231	Transposase	2658997 _ 2658308	-0.41	0.34
MM_2232	Transposase	2659481 _ 2659155	-0.15	0.49
MM_2235	Conserved protein	2665106 _ 2666461	-0.54	0.38
MM_2237	Transcriptional regulator, ArsR family	2668004 _ 2667666	0.61	0.23
MM_2240	Conserved protein	2669541 _ 2670119	-0.07	0.07
MM_2245	Conserved protein	2673772 _ 2672876	0.47	0.21
MM_2248	Conserved protein	2676033 _ 2677001	-0.06	0.39
MM_2249	Hypothetical protein	2677089 _ 2677316	-0.21	0.24
MM_2252	Conserved protein	2679852 _ 2678455	-0.22	0.22
MM_2255	Conserved protein	2683082 _ 2682534	0.08	0.28
MM_2256	Conserved protein	2684062 _ 2683367	0.27	0.41
MM_2258	Conserved protein	2685333 _ 2685040	-0.03	0.31
MM_2260	Hypothetical protein	2686159 _ 2685719	-0.22	0.26
MM_2261	Conserved protein	2686940 _ 2686137	0.00	0.46
MM_2263	SSU ribosomal protein S10P	2689028 _ 2688723	0.20	0.44
MM_2264	Protein translation elongation factor 1A (EF-1A)	2690335 _ 2689070	0.24	0.26
MM_2265	Protein translation elongation factor 2	2692882 _ 2690693	-0.26	0.52
MM_2266	SSU ribosomal protein S7P	2693515 _ 2692949	-0.21	0.46
MM_2267	SSU ribosomal protein S12P	2693940 _ 2693515	-0.41	0.43
MM_2268	NusA protein homolog	2694471 _ 2694049	-0.39	0.35
MM_2269	LSU ribosomal protein L30E	2694803 _ 2694495	-0.19	0.25
MM_2270	DNA-directed RNA polymerase, su A''	2696264 _ 2695041	-0.23	0.19
MM_2271	DNA-directed RNA polymerase, su A'	2698903 _ 2696264	-0.09	0.18
MM_2272	DNA-directed RNA polymerase, su B''	2700731 _ 2698920	-0.34	0.44
MM_2273	DNA-directed RNA polymerase, su B'	2702341 _ 2700749	-0.45	0.22
MM_2274	DNA-directed RNA polymerase, su H	2702884 _ 2702564	-0.39	0.27
MM_2275	Hypothetical sensory transduction histidine kinase	2707000 _ 2703488	-0.06	0.32
MM_2276	Hypothetical sensory transduction histidine kinase	2710908 _ 2707588	-0.02	0.22
MM_2278	Adenosylhomocysteinase	2717212 _ 2715980	-0.10	0.20
MM_2279	Chlorohydrolase family protein	2718874 _ 2717504	-0.20	0.39
MM_2280	Conserved protein	2720016 _ 2719366	0.18	0.40
MM_2281	Hypothetical protein	2720808 _ 2720050	-0.09	0.57
MM_2283	DNA/pantothenate metabolism flavoprotein	2723700 _ 2722303	0.31	0.28
MM_2284	Conserved protein	2724078 _ 2724626	-0.17	0.39
MM_2285	Conserved protein	2724553 _ 2724335	0.16	0.62
MM_2286	Conserved protein	2725064 _ 2725957	0.21	0.26
MM_2287	Hypothetical protein	2726470 _ 2727300	-0.26	0.21
MM_2288	Methyltranferase involved in cell division	2727361 _ 2728164	-0.60	0.26
MM_2289	Hypothetical protein	2728321 _ 2728962	-0.08	0.46

MM_2290	Cell surface protein	2729681 _ 2731051	-0.42	0.15
MM_2291	Zinc metalloprotease	2732159 _ 2731446	-0.03	0.37
MM_2292	Type I restriction-modification system restriction su	2735287 _ 2732162	-0.16	0.23
MM_2293	Type I restriction-modification system specificity su	2736504 _ 2735287	-0.25	0.33
MM_2294	Type I restriction-modification system methylation su	2738927 _ 2736504	0.22	0.32
MM_2296	Conserved protein	2743987 _ 2742812	0.26	0.17
MM_2297	Conserved protein	2746356 _ 2744422	-0.01	0.35
MM_2299	Hypothetical protein	2750714 _ 2749530	0.09	0.57
MM_2301	Carbon monoxide dehydrogenase	2753816 _ 2751915	0.55	0.40
MM_2302	Conserved protein	2754351 _ 2755610	-0.21	0.45
MM_2303	Transcriptional regulator	2755749 _ 2757044	-0.18	0.15
MM_2305	Sodium/proline symporter	2759796 _ 2758135	-0.33	0.80
MM_2306	L-asparaginase	2761877 _ 2760603	0.08	0.40
MM_2307	Argininosuccinate lyase	2763669 _ 2762197	-0.31	0.27
MM_2309	Hypothetical protein	2765502 _ 2764570	0.49	0.33
MM_2312	F420-nonreducing hydrogenase I, cytochrome b su (VhoC)	2769098 _ 2768286	0.18	0.45
MM_2313	F420-nonreducing hydrogenase I, large su (VhoA)	2770899 _ 2769127	-0.23	0.67
MM_2314	F420-nonreducing hydrogenase I, small su (VhoG)	2772067 _ 2770910	-0.10	0.50
MM_2315	Hydrogenase expression/formation protein	2773179 _ 2773436	0.04	0.20
MM_2316	Hydrogenase expression/formation protein	2773442 _ 2774575	-0.19	0.42
MM_2317	Hydrogenase expression/formation protein	2774571 _ 2774981	0.05	0.37
MM_2320	Ech Hydrogenase, su A	2778411 _ 2780324	0.00	0.44
MM_2321	Ech Hydrogenase, su B	2780327 _ 2781196	-0.35	0.41
MM_2322	Ech Hydrogenase, su C	2781213 _ 2781680	-0.09	0.69
MM_2323	Ech Hydrogenase, su D	2781676 _ 2782014	-0.11	0.73
MM_2324	Ech Hydrogenase, su E	2782040 _ 2783113	-0.12	0.30
MM_2325	Ech Hydrogenase, su F	2783126 _ 2783503	0.08	0.67
MM_2326	COP associated protein	2784313 _ 2784531	0.21	0.29
MM_2328	Copper-exporting ATPase	2784953 _ 2787838	0.10	0.33
MM_2330	Hypothetical protein	2788227 _ 2788418	-0.17	0.63
MM_2331	Uncharacterized permease	2789837 _ 2788494	-0.45	0.12
MM_2332	SAM-dependent methyltransferases	2790987 _ 2791667	0.04	0.08
MM_2333	Multidrug efflux pump	2792142 _ 2791819	0.20	0.46
MM_2334	Conserved protein	2792632 _ 2792871	0.09	0.22
MM_2335	Conserved protein	2793152 _ 2793412	-0.24	0.49
MM_2337	Conserved protein	2794761 _ 2795117	0.16	0.38
MM_2338	Conserved protein in Methanosarcina spec.	2795360 _ 2795557	-0.35	0.16
MM_2339	Translation initiation factor 1A (EIF-1A)	2796138 _ 2796314	-0.42	0.41
MM_2340	Acetolactate synthase	2797986 _ 2796355	0.09	0.84
MM_2341	Succinate-semialdehyde dehydrogenase [NADP+]	2799521 _ 2798160	-0.17	0.32
MM_2342	Hypothetical protein	2799785 _ 2800210	0.11	0.10
MM_2344	Copper-binding protein	2801517 _ 2802071	0.04	0.18
MM_2346	Methyltransferase	2804059 _ 2804895	0.06	0.55
MM_2348	O-linked N-acetyglucosamine transferase	2807529 _ 2806294	0.04	0.48
MM_2349	Putative phosphatase	2807738 _ 2808529	0.01	0.08
MM_2350	O-linked N-acetyglucosamine transferase	2809748 _ 2808873	0.46	0.21
MM_2351	Transcriptional regulator	2811230 _ 2810721	0.26	0.65
MM_2353	Thioredoxin reductase	2813001 _ 2812087	-0.06	0.72
MM_2354	Thioredoxin	2813393 _ 2813058	0.18	0.69
MM_2355	Diphthine synthase	2813461 _ 2814258	-0.12	0.27
MM_2357	Hypothetical protein	2814849 _ 2815235	-0.38	0.19
MM_2358	6-Phosphofructokinase (ADP)	2815505 _ 2816401	-0.20	0.32
MM_2359	Conserved protein	2816531 _ 2817205	-0.17	0.31
MM_2360	Phosphoribosylaminoimidazole carboxylase, catalytic su	2818071 _ 2817679	0.15	0.51
MM_2361	Chorismate mutase / prephenate dehydratase	2818380 _ 2818096	0.26	0.31
MM_2362	Shikimate kinase	2819258 _ 2818380	0.25	0.49
MM_2363	Conserved protein	2819580 _ 2820497	-0.19	0.38
MM_2364	GTP-binding protein	2821477 _ 2822145	-0.19	0.49
MM_2365	Conserved protein	2822154 _ 2822510	-0.33	0.37

MM_2366	Conserved protein	2822527 _ 2823036	-0.19	0.39
MM_2367	Hexulose-6-phosphate isomerase	2823290 _ 2823946	-0.11	0.50
MM_2368	Aspartate aminotransferase	2824002 _ 2825198	0.46	0.42
MM_2369	Cytochrome c-type biogenesis protein	2825620 _ 2826480	0.00	0.53
MM_2370	GTP-binding protein	2827609 _ 2826596	-0.40	0.26
MM_2371	Conserved protein	2828110 _ 2828982	0.40	0.33
MM_2373	3-Isopropylmalate dehydratase	2830953 _ 2829595	-0.12	0.49
MM_2374	Conserved protein	2831886 _ 2831047	0.16	0.44
MM_2375	Conserved protein	2832077 _ 2832577	-0.47	0.65
MM_2376	Conserved protein	2834169 _ 2833429	0.19	0.47
MM_2378	Conserved transmembrane protein	2835346 _ 2836479	0.25	0.60
MM_2379	5'-Methylthioadenosine phosphorylase	2836547 _ 2837320	-0.02	0.11
MM_2380	ATP-dependent DNA helicase	2838031 _ 2840508	-0.06	0.21
MM_2382	Hypothetical protein	2841298 _ 2841573	0.02	0.74
MM_2383	Small nuclear riboprotein (snRNA) homolog	2841563 _ 2841784	-0.02	0.52
MM_2384	Conserved protein	2841817 _ 2842239	-0.15	0.40
MM_2387	Cobalt transport ATP-binding protein CbiO	2845226 _ 2846719	0.25	0.61
MM_2388	Cobalt ABC transporter, permease protein CbiQ	2846719 _ 2847504	0.10	0.56
MM_2392	Long-chain-fatty-acid--CoA ligase	2855089 _ 2853455	-0.03	0.55
MM_2393	Conserved protein	2855641 _ 2855189	0.10	0.47
MM_2394	Transcriptional regulator, MerR family	2856330 _ 2855755	0.08	0.61
MM_2396	Heme exporter, protein C	2858672 _ 2857962	0.17	0.28
MM_2397	Heme exporter, protein B	2859756 _ 2859079	0.19	0.52
MM_2398	ABC transporter, ATP-binding protein	2860649 _ 2859939	0.29	0.42
MM_2399	Peptide methionine sulfoxide reductase	2861454 _ 2860822	0.16	0.67
MM_2400	Hypothetical protein	2862134 _ 2861553	0.20	0.45
MM_2402	Hypothetical protein	2863730 _ 2862855	0.40	0.44
MM_2403	Potassium channel protein	2865448 _ 2864252	0.24	0.31
MM_2405	Cation transporter	2866801 _ 2866178	0.30	0.46
MM_2406	Conserved protein	2867470 _ 2868165	-0.36	0.20
MM_2407	Conserved protein	2868971 _ 2869633	-0.39	0.26
MM_2409	Conserved protein	2870639 _ 2871535	0.04	0.31
MM_2415	Hypothetical protein	2876372 _ 2876136	-0.09	0.55
MM_2416	Transcriptional regulator, ASNC family	2876691 _ 2876900	-0.08	0.50
MM_2417	Type II DNA topoisomerase VI, su B	2877339 _ 2879201	-0.24	0.28
MM_2419	DNA gyrase, su B	2881210 _ 2883111	-0.12	0.62
MM_2420	DNA gyrase, su A	2883276 _ 2885975	-0.20	0.74
MM_2421	Chaperone protein	2886629 _ 2889574	-0.09	0.19
MM_2422	Conserved protein	2890766 _ 2889711	0.01	0.45
MM_2424	Conserved protein	2893501 _ 2892347	-0.08	0.09
MM_2428	Methylthiol:coenzyme M methyltransferase MtsB	2897978 _ 2898802	0.39	0.36
MM_2431	Conserved protein	2900807 _ 2901577	0.07	0.17
MM_2432	Putative pyridoxine biosynthesis protein	2901968 _ 2902870	-0.26	0.50
MM_2433	Imidazoleglycerol-phosphate synthase	2903175 _ 2903783	-0.25	0.35
MM_2435	Hypothetical sensory transduction histidine kinase	2906493 _ 2909147	-0.12	0.22
MM_2438	Hypothetical protein	2912093 _ 2912497	0.26	0.55
MM_2442	Conserved protein	2915444 _ 2916169	0.00	0.34
MM_2444	Conserved protein	2917371 _ 2917853	-0.25	0.16
MM_2445	Conserved protein	2918091 _ 2918951	-0.24	0.19
MM_2446	Transcriptional regulator, ArsR family	2919355 _ 2920068	-0.30	0.82
MM_2448	Transposase	2920854 _ 2921510	-0.05	0.55
MM_2449	Conserved protein	2921987 _ 2922550	-0.27	0.27
MM_2451	Conserved protein	2924441 _ 2925055	-0.24	0.30
MM_2452	Conserved protein	2925549 _ 2926385	-0.22	0.38
MM_2453	Conserved protein	2926722 _ 2927378	-0.02	0.23
MM_2454	ABC transporter, permease protein	2928652 _ 2927891	-0.15	0.46
MM_2455	ABC transporter, ATP-binding protein	2929659 _ 2928652	-0.02	0.36
MM_2457	Dipeptide/oligopeptide transporter, permease protein	2932531 _ 2931605	-0.21	0.84
MM_2458	Dipeptide/oligopeptide transporter, permease protein	2933511 _ 2932531	-0.14	0.61
MM_2459	Methyltransferase	2934402 _ 2933542	-0.13	0.62

MM_2460	Dipeptide/oligopeptide-binding protein	2936059 _ 2934383	-0.23	0.49
MM_2463	Protein translation initiation factor IF2	2939673 _ 2937901	-0.46	0.25
MM_2464	Nucleoside diphosphate kinase	2940313 _ 2939834	-0.27	0.27
MM_2466	SSU ribosomal protein S28E	2940743 _ 2940525	-0.12	0.40
MM_2467	LSU ribosomal protein L7AE	2941130 _ 2940771	-0.27	0.54
MM_2468	Metallo cofactor biosynthesis protein (moaA/ nifB /pqqE family	2941441 _ 2942091	-0.28	0.14
MM_2469	Hypothetical protein	2943768 _ 2942287	-0.13	0.84
MM_2470	Surface layer protein (putative)	2949026 _ 2944008	0.05	0.56
MM_2471	Hypothetical protein	2951238 _ 2949937	0.19	0.57
MM_2477	Hypothetical protein	2955178 _ 2954684	0.27	0.50
MM_2478	Chloride channel (putative)	2958365 _ 2955354	-0.44	0.19
MM_2479	F420H2 dehydrogenase, suO	2959379 _ 2958987	-0.42	0.05
MM_2480	F420H2 dehydrogenase, suN	2960874 _ 2959396	-0.03	0.11
MM_2481	F420H2 dehydrogenase, suM	2962365 _ 2960881	-0.12	0.13
MM_2482	F420H2 dehydrogenase, suL	2964383 _ 2962368	-0.05	0.53
MM_2483	F420H2 dehydrogenase, suK	2964685 _ 2964380	-0.36	0.29
MM_2484	F420H2 dehydrogenase, suJ2	2964933 _ 2964685	0.03	0.40
MM_2486	F420H2 dehydrogenase, suI	2965743 _ 2965213	-0.02	0.07
MM_2487	F420H2 dehydrogenase, suH	2966675 _ 2965626	-0.07	0.20
MM_2488	F420H2 dehydrogenase, suD	2967796 _ 2966675	-0.12	0.11
MM_2489	F420H2 dehydrogenase, suC	2968283 _ 2967810	-0.40	0.07
MM_2490	F420H2 dehydrogenase, suB	2968867 _ 2968316	0.11	0.63
MM_2491	F420H2 dehydrogenase, suA	2969244 _ 2968858	0.04	0.72
MM_2493	Oxidoreductase (hypothetical)	2970408 _ 2971682	0.08	0.08
MM_2494	FO synthase, su CofG	2971708 _ 2972745	0.04	0.67
MM_2495	F420 biosynthesis protein FbiC	2972745 _ 2973872	-0.05	0.47
MM_2496	FO synthase, su CofH	2973907 _ 2975082	0.15	0.68
MM_2497	4-Methyl-5(B-hydroxyethyl)-thiazole monophosphate synthase	2975181 _ 2975804	-0.07	0.56
MM_2498	Conserved protein	2976310 _ 2976741	0.22	0.61
MM_2499	Geranylgeranyl reductase	2977687 _ 2978937	0.03	0.50
MM_2500	Trk system potassium uptake protein	2980515 _ 2979088	0.32	0.56
MM_2501	Trk system potassium uptake protein	2982092 _ 2980668	-0.11	0.30
MM_2502	Trk system potassium uptake protein	2983734 _ 2982307	0.00	0.59
MM_2503	Trk system potassium uptake protein TrkA	2985122 _ 2983779	-0.46	0.30
MM_2504	Chaperone protein DnaJ	2986824 _ 2985658	-0.22	0.23
MM_2505	Chaperone protein	2988794 _ 2986938	0.23	0.43
MM_2506	GrpE protein	2989798 _ 2989199	0.16	0.35
MM_2508	Heat shock protein	2991856 _ 2991458	0.26	0.41
MM_2510	Hypothetical protein	2994006 _ 2994959	-0.38	0.31
MM_2511	Hypothetical protein	2994949 _ 2996025	-0.22	0.09
MM_2512	1-Pyrroline-5-carboxylate synthetase	2996177 _ 2996842	0.16	0.38
MM_2514	Archaeal protein Translation Elongation Factor 1, su beta	2997299 _ 2997565	0.21	0.29
MM_2515	Hypothetical sensory transduction histidine kinase	2998422 _ 3001571	0.05	0.47
MM_2516	Probable transcriptional regulator	3001574 _ 3001984	0.18	0.60
MM_2517	Thiamine biosynthesis protein	3003367 _ 3002153	-0.26	0.31
MM_2519	GTP-binding protein homolog	3006525 _ 3007286	0.17	0.17
MM_2520	Conserved protein	3008236 _ 3007868	0.45	0.16
MM_2522	Universal stress protein	3009155 _ 3009601	-0.38	0.34
MM_2523	Hypothetical protein	3010179 _ 3009904	0.38	0.35
MM_2524	Phage shock protein A	3010940 _ 3010185	0.04	0.41
MM_2525	Hypothetical protein	3011382 _ 3012119	-0.38	0.35
MM_2526	Conserved protein	3012985 _ 3012293	0.17	0.45
MM_2527	Hypothetical protein	3013693 _ 3013031	-0.03	0.59
MM_2528	N2,N2-dimethylguanosine tRNA methyltransferase	3014985 _ 3013822	0.04	0.31
MM_2529	Hypothetical protein	3015423 _ 3015013	-0.19	0.31
MM_2530	Transposase	3017059 _ 3015884	0.02	0.19
MM_2531	Hypothetical protein	3017885 _ 3017439	0.09	0.56
MM_2532	Hypothetical protein	3019888 _ 3018650	0.00	0.17
MM_2533	Hypothetical protein	3021033 _ 3020350	0.10	0.09

MM_2534	Conserved hypothetical protein	3021179 _ 3021811	-0.10	0.33
MM_2535	Conserved hypothetical protein	3022074 _ 3023366	-0.31	0.15
MM_2536	LSU ribosomal protein L21E	3023622 _ 3023912	-0.42	0.24
MM_2537	Hypothetical protein	3024386 _ 3024736	-0.10	0.71
MM_2538	Conserved hypothetical protein	3025079 _ 3025807	-0.24	0.47
MM_2540	Conserved hypothetical protein	3026739 _ 3027344	-0.17	0.40
MM_2542	Phosphohydrolase (MUTT/NUDIX family protein)	3030526 _ 3031380	0.09	0.60
MM_2547	Putative ferredoxin	3033943 _ 3033146	0.01	0.17
MM_2550	Hypothetical protein	3035493 _ 3035257	-0.01	0.78
MM_2552	Hypothetical protein	3037056 _ 3036424	-0.18	0.26
MM_2554	Acetyltransferase	3038572 _ 3039222	-0.66	0.32
MM_2556	ATP-dependent RNA helicase	3039757 _ 3041043	-0.51	0.35
MM_2557	Catalase	3041787 _ 3043481	-0.07	0.68
MM_2558	Hypothetical protein	3043997 _ 3044983	0.03	0.39
MM_2559	Hypothetical protein	3045342 _ 3046475	-0.15	0.45
MM_2561	Conserved hypothetical protein	3047545 _ 3048639	-0.12	0.77
MM_2562	Hypothetical protein	3048799 _ 3051369	-0.20	0.48
MM_2563	Ferredoxin	3051849 _ 3052955	0.20	0.11
MM_2564	Hypothetical protein	3053488 _ 3053129	-0.07	0.46
MM_2565	Pyruvate formate-lyase activating enzyme	3053666 _ 3054700	-0.17	0.34
MM_2566	CODH nickel-insertion accessory protein CooC	3055158 _ 3055919	-0.42	0.36
MM_2567	ABC transporter, periplasmic binding protein	3057354 _ 3058925	-0.16	0.65
MM_2568	ABC transporter, permease protein	3059042 _ 3059980	-0.03	0.74
MM_2569	ABC transporter, permease protein	3059967 _ 3060863	-0.05	0.54
MM_2570	ABC transporter, ATP-binding protein	3060863 _ 3061888	-0.20	0.81
MM_2571	ABC transporter, ATP-binding protein	3061879 _ 3062574	-0.30	0.65
MM_2572	Methyltransferase	3062742 _ 3063965	-0.31	0.18
MM_2573	Iron dependent transcriptional repressor	3064700 _ 3064218	0.57	0.22
MM_2580	Hypothetical protein	3070258 _ 3070004	-0.05	0.39
MM_2581	Pyruvate formate-lyase activating enzyme related protein	3070278 _ 3071327	0.29	0.22
MM_2582	Hypothetical protein	3071946 _ 3071437	-0.20	0.34
MM_2583	Sec-independent protein translocase, protein	3072435 _ 3072133	-0.26	0.42
MM_2584	Sec-independent protein translocase protein TatA	3073088 _ 3072723	-0.02	0.56
MM_2585	Sec-independent protein translocase, protein	3073994 _ 3073221	-0.02	0.48
MM_2586	Sec-independent protein translocase, protein	3074752 _ 3075510	0.05	0.56
MM_2587	Surface layer protein (putative)	3078031 _ 3075767	-0.27	0.09
MM_2592	Hypothetical protein	3082413 _ 3088106	-0.15	0.40
MM_2593	Hypothetical protein	3088329 _ 3089300	-0.09	0.27
MM_2594	Hypothetical protein	3089484 _ 3090641	0.30	0.32
MM_2595	Hypothetical protein	3091355 _ 3090807	0.08	0.41
MM_2596	Hypothetical protein	3093621 _ 3091627	0.58	0.30
MM_2597	Hypothetical protein	3094307 _ 3093789	0.29	0.20
MM_2598	Hypothetical protein	3095704 _ 3094331	0.17	0.68
MM_2603	Hypothetical protein	3100703 _ 3100281	0.24	0.50
MM_2605	Conserved protein	3102529 _ 3102804	0.04	0.54
MM_2608	Nucleotidyltransferase	3105115 _ 3104798	0.44	0.42
MM_2610	Type I restriction enzyme	3106687 _ 3105791	-0.03	0.38
MM_2612	Probable ATP-dependent helicase	3113587 _ 3110852	-0.24	0.44
MM_2613	LemA protein	3114614 _ 3114111	0.26	0.78
MM_2614	Hypothetical protein	3116618 _ 3114768	0.18	0.26
MM_2615	Hypothetical protein	3118887 _ 3116878	0.25	0.49
MM_2616	LSU ribosomal protein L15E	3119379 _ 3119975	-0.15	0.16
MM_2617	Hypothetical protein	3119975 _ 3120415	-0.14	0.47
MM_2618	Hypothetical protein	3120411 _ 3121127	-0.37	0.46
MM_2620	Proteasome, su-alpha	3122919 _ 3123665	0.12	0.22
MM_2621	Hypothetical protein	3123747 _ 3124436	0.03	0.64
MM_2622	Hypothetical protein	3124451 _ 3125230	-0.14	0.30
MM_2623	Ribonuclease	3125312 _ 3126790	-0.20	0.43
MM_2624	Ribonuclease	3126777 _ 3127574	0.16	0.43
MM_2625	LSU ribosomal protein L37AE	3127741 _ 3128022	0.12	0.30

MM_2628	Conserved protein	3129580 _ 3129825	0.45	0.22
MM_2634	2-Keto acid:ferredoxin oxidoreductase su alpha	3140930 _ 3142798	-0.04	0.17
MM_2635	Putative pyruvate:ferredoxin oxidoreductase	3142798 _ 3143487	0.27	0.50
MM_2636	Coenzyme F390 synthetase/phenylacetyl-CoA ligase	3143483 _ 3144787	0.18	0.39
MM_2637	Hypothetical protein	3146321 _ 3145212	-0.05	0.48
MM_2638	Hypothetical protein	3148832 _ 3146676	0.09	0.24
MM_2639	Conserved protein	3151348 _ 3150431	0.00	0.47
MM_2641	Conserved protein	3153400 _ 3154140	-0.07	0.26
MM_2645	Aldehyde ferredoxin oxidoreductase, tungsten-containing	3158129 _ 3156306	0.18	0.20
MM_2646	Sensory Transduction protein Kinase	3161120 _ 3158472	-0.10	0.45
MM_2648	Hypothetical protein	3164058 _ 3163291	0.05	0.45
MM_2649	Aspartate aminotransferase	3165503 _ 3164337	-0.03	0.27
MM_2650	Hydrolase	3165905 _ 3166696	0.13	0.17
MM_2652	Hypothetical protein	3168107 _ 3167643	0.10	0.26
MM_2653	N5,N10-methenyltetrahydromethanopterin cyclohydrolase	3169299 _ 3168337	-0.27	0.50
MM_2654	Hypothetical protein	3170853 _ 3169660	-0.50	0.09
MM_2656	Peptidyl-prolyl cis-trans isomerase	3175080 _ 3174331	0.10	0.42
MM_2657	transcriptional regulator	3175787 _ 3176206	-0.22	0.70
MM_2658	Acetyltransferase	3176501 _ 3177067	-0.32	0.51
MM_2659	Acetyltransferase	3177337 _ 3177849	-0.10	0.43
MM_2660	Sodium-calcium exchanger	3178006 _ 3179181	0.18	0.53
MM_2661	Hypothetical protein	3179701 _ 3179276	0.00	0.24
MM_2663	Conserved protein	3181471 _ 3182742	0.54	0.51
MM_2664	Conserved protein	3182808 _ 3184010	0.45	0.30
MM_2665	ABC transporter, ATP-binding protein	3184026 _ 3184820	0.16	0.31
MM_2666	Conserved protein	3185349 _ 3186173	0.21	0.33
MM_2674	Conserved protein	3199650 _ 3196663	-0.07	0.24
MM_2675	Serine protease inhibitor	3201042 _ 3199765	0.01	0.33
MM_2676	Conserved protein	3201412 _ 3202749	0.22	0.58
MM_2679	Conserved protein	3204091 _ 3204951	-0.11	0.17
MM_2680	Conserved protein	3205377 _ 3205120	0.71	0.09
MM_2683	Reverse transcriptase	3207154 _ 3208650	-0.11	0.20
MM_2687	Hypothetical protein	3211923 _ 3210985	-0.08	0.60
MM_2692	Hypothetical protein	3217665 _ 3216277	-0.23	0.65
MM_2693	Hypothetical protein	3218252 _ 3219304	-0.15	0.31
MM_2695	Reverse transcriptase	3221510 _ 3222118	-0.23	0.70
MM_2698	Reverse transcriptase	3223005 _ 3223754	-0.36	0.29
MM_2699	Transposase	3223789 _ 3224535	0.40	0.44
MM_2700	Transposase	3224535 _ 3225329	0.07	0.08
MM_2703	Type I restriction-modification system specificity su	3231517 _ 3230198	-0.10	0.32
MM_2704	Type I restriction-modification system methylation su	3232925 _ 3231504	-0.01	0.76
MM_2705	Type I restriction-modification system restriction su	3235603 _ 3232925	0.11	0.33
MM_2706	Conserved protein	3236326 _ 3236649	-0.07	0.74
MM_2709	Conserved protein	3239061 _ 3238774	-0.19	0.48
MM_2710	Conserved protein	3240255 _ 3239212	-0.17	0.11
MM_2711	Conserved protein	3240471 _ 3241667	0.09	0.18
MM_2712	Conserved protein	3242109 _ 3242639	-0.14	0.25
MM_2713	Homoserine dehydrogenase	3242664 _ 3243659	-0.33	0.40
MM_2714	ATP-dependent DNA ligase	3243731 _ 3245434	-0.29	0.31
MM_2715	Hypothetical protein	3246136 _ 3245510	-0.12	0.36
MM_2716	Conserved protein	3247122 _ 3246136	-0.23	0.35
MM_2717	Conserved protein	3249269 _ 3247668	0.12	0.22
MM_2718	Hypothetical protein	3249809 _ 3250726	-0.06	0.24
MM_2719	Conserved protein	3251581 _ 3250796	-0.21	0.54
MM_2721	Conserved protein	3256304 _ 3256029	0.30	0.52
MM_2722	Hypothetical protein	3256475 _ 3256957	-0.16	0.18
MM_2723	Phosphoenolpyruvate synthase	3257182 _ 3259596	0.11	0.56
MM_2727	Conserved protein	3262044 _ 3263336	-0.42	0.22
MM_2731	Multiple antibiotic resistance protein	3269239 _ 3268604	0.06	0.55

MM_2732	Conserved protein	3270181 _ 3269561	0.45	0.25
MM_2736	Conserved protein	3274913 _ 3274365	-0.29	0.46
MM_2737	Conserved protein	3275709 _ 3275119	-0.35	0.13
MM_2739	Type I restriction-modification system specificity su	3277544 _ 3276327	-0.28	0.90
MM_2740	Type I restriction-modification system methylation su	3279070 _ 3277547	-0.24	0.29
MM_2741	Hypothetical protein	3280541 _ 3279450	0.08	0.89
MM_2742	Hypothetical protein	3282873 _ 3280570	0.14	0.25
MM_2744	Conserved protein	3285082 _ 3283994	0.35	0.45
MM_2745	Conserved protein	3286436 _ 3285318	0.20	0.19
MM_2746	Conserved protein	3288189 _ 3286939	0.08	0.31
MM_2747	Hypothetical protein	3288741 _ 3289964	-0.27	0.22
MM_2748	Hypothetical sensory transduction histidine kinase	3290910 _ 3293795	0.28	0.31
MM_2751	Conserved protein	3296786 _ 3296301	-0.10	0.49
MM_2758	Hypothetical protein	3305933 _ 3306460	-0.10	0.69
MM_2769	Iron-containing alcohol dehydrogenase	3320558 _ 3319287	0.09	0.54
MM_2770	Glycogen debranching enzyme	3320721 _ 3322697	0.11	0.55
MM_2772	Conserved protein	3324088 _ 3324366	-0.19	0.57
MM_2773	Two component system histidine kinase	3327153 _ 3324733	0.09	0.40
MM_2774	Conserved protein	3327606 _ 3328088	0.22	0.21
MM_2775	Conserved protein	3328177 _ 3329076	0.52	0.35
MM_2777	Acylphosphatase	3329961 _ 3330239	0.19	0.48
MM_2782	Glyceraldehyde 3-phosphate dehydrogenase	3335707 _ 3336711	-0.13	0.49
MM_2783	Suppressor protein SuhB homolog	3336891 _ 3337691	0.15	0.25
MM_2784	Hypothetical protein	3337654 _ 3338520	-0.09	0.42
MM_2785	2-Isopropylmalate synthase	3338845 _ 3340194	0.19	0.32
MM_2786	Conserved protein	3340890 _ 3340408	0.19	0.44
MM_2788	Indolepyruvate oxidoreductase, alpha su	3346268 _ 3344520	-0.13	0.15
MM_2789	Hypothetical protein	3347374 _ 3346337	-0.33	0.11
MM_2792	Molybdopterin converting factor, su 2	3349614 _ 3350447	0.27	0.44
MM_2793	Molybdopterin-guanine dinucleotide biosynthesis protein	3350576 _ 3351247	0.14	0.40
MM_2794	Hypothetical protein	3352031 _ 3351459	0.04	0.14
MM_2795	Conserved protein	0	-0.07	0.71
MM_2798	Conserved protein	3357284 _ 3358690	0.13	0.20
MM_2800	Conserved protein	3359988 _ 3360494	-0.10	0.41
MM_2802	Hypothetical protein	3361979 _ 3362551	0.05	0.44
MM_2803	Carbon monoxide dehydrogenase accessory protein	3363344 _ 3362682	0.47	0.31
MM_2805	Asparagine synthetase [glutamine-hydrolyzing]	3367720 _ 3366242	-0.11	0.48
MM_2806	Hypothetical protein	3368918 _ 3369166	-0.14	0.55
MM_2808	Conserved protein	3371716 _ 3370601	0.04	0.61
MM_2812	Phenylalanyl-tRNA synthetase, beta chain	3376311 _ 3374677	0.19	0.25
MM_2813	Hypothetical protein	3376602 _ 3377354	0.30	0.08
MM_2815	Metal-dependent hydrolase	3379088 _ 3378249	-0.17	0.58
MM_2816	Conserved protein	3379428 _ 3380171	0.25	0.25
MM_2820	Anthranilate phosphoribosyltransferase	3384599 _ 3383490	0.28	0.66
MM_2824	Coenzyme F420-dependent glucose-6-phosphate dehydrogenase	3388292 _ 3389089	-0.16	0.45
MM_2829	Peptidyl-prolyl cis-trans isomerase	3393854 _ 3394318	0.24	0.21
MM_2832	ABC transporter, permease protein	3397793 _ 3398707	-0.19	0.59
MM_2833	ABC transporter, ATP-binding protein	3398933 _ 3399613	0.08	0.42
MM_2835	Hypothetical protein	3400631 _ 3400245	0.50	0.42
MM_2836	Enolase	3401314 _ 3402597	0.17	0.46
MM_2838	Conserved protein	3404345 _ 3404100	0.19	0.53
MM_2839	MoxR-like ATPase	3404768 _ 3406360	0.10	0.24
MM_2841	Acetolactate synthase	3410114 _ 3408363	0.13	0.46
MM_2843	Hypothetical protein	3411741 _ 3412976	0.22	0.29
MM_2845	Hypothetical protein	3414977 _ 3414576	0.00	0.31
MM_2846	Putrescine-ornithine antiporter	3416637 _ 3415264	0.46	0.27
MM_2847	Amino acid permease	3418092 _ 3416746	-0.04	0.61
MM_2848	Conserved protein	3420021 _ 3419383	0.01	0.31
MM_2849	Hypothetical permease	3420445 _ 3421254	-0.18	0.18

MM_2850	ABC transporter, permease protein	3422563 _ 3421349	0.06	0.34
MM_2851	Transcriptional regulator, MarR family	3423038 _ 3423520	0.35	0.37
MM_2853	Hypothetical protein	3426755 _ 3427228	-0.36	0.52
MM_2855	ABC transporter, permease protein	3428574 _ 3429413	0.07	0.51
MM_2858	Potassium channel protein	3432177 _ 3432596	-0.12	0.70
MM_2859	Hypothetical protein	3434424 _ 3433576	0.05	0.29
MM_2860	Conserved protein	3434958 _ 3434722	0.23	0.47
MM_2862	Hypothetical protein	3437892 _ 3437047	0.04	0.51
MM_2864	Conserved protein	3438733 _ 3439626	-0.23	0.55
MM_2867	Coenzyme F390 synthetase/phenylacetyl-CoA ligase	3445863 _ 3444565	-0.39	0.55
MM_2869	Hypothetical protein	3448149 _ 3448781	0.22	0.27
MM_2871	Hypothetical protein	3449557 _ 3449829	-0.24	0.23
MM_2872	Methyltransferase	3451119 _ 3450838	-0.08	0.51
MM_2874	Conserved protein	3452831 _ 3452418	0.06	0.51
MM_2875	Hypothetical protein	3453320 _ 3453973	0.03	0.13
MM_2876	DNA polymerase IV	3454126 _ 3455232	0.01	0.33
MM_2877	Hypothetical protein	3455326 _ 3456366	0.49	0.42
MM_2878	Hypothetical protein	3457408 _ 3456680	0.31	0.51
MM_2879	Tetratrico peptide repeat protein	3458794 _ 3457748	-0.27	0.49
MM_2882	Conserved protein	3464721 _ 3463627	0.16	0.37
MM_2883	Hypothetical protein	3465701 _ 3465021	-0.06	0.33
MM_2884	Conserved protein	3466814 _ 3465822	0.18	0.57
MM_2885	Metalloendopeptidases (putative)	3468039 _ 3467437	-0.20	0.10
MM_2886	Hypothetical sensory transduction histidine kinase	3471083 _ 3469080	0.20	0.26
MM_2888	Endonuclease III	3472911 _ 3472168	0.00	0.54
MM_2891	ABC transporter, permease protein	3474653 _ 3473829	-0.25	0.58
MM_2892	ABC transporter, permease protein	3475184 _ 3474735	-0.31	0.98
MM_2893	Hypothetical protein	3476562 _ 3475213	-0.30	0.50
MM_2899	Conserved protein	3483255 _ 3482479	-0.09	0.70
MM_2900	Conserved protein	3484496 _ 3483459	0.46	0.42
MM_2903	Transposase	3486296 _ 3487393	0.21	0.11
MM_2908	Hypothetical protein	3491164 _ 3491853	0.01	0.80
MM_2910	Adenine deaminase	3495354 _ 3493690	0.06	0.43
MM_2911	Phosphoserine aminotransferase	3495669 _ 3496778	0.26	0.15
MM_2912	5-Oxoprolinase	3497146 _ 3500895	0.32	0.37
MM_2913	Conserved protein	3501997 _ 3500930	0.29	0.33
MM_2914	Conserved protein	3502995 _ 3502492	-0.05	0.47
MM_2915	Hypothetical protein	3503626 _ 3503018	-0.02	0.65
MM_2916	Flavoprotein	3504011 _ 3504574	0.31	0.26
MM_2917	Oxidoreductase, aldo/keto family	3506220 _ 3505027	0.08	0.29
MM_2918	Oxidoreductase, aldo/keto family	3507517 _ 3506339	0.28	0.70
MM_2919	Hypothetical protein	3509113 _ 3508787	0.28	0.45
MM_2924	Ribonuclease BN	3518430 _ 3517567	-0.15	0.47
MM_2925	Hypothetical protein	3519244 _ 3518945	0.13	0.44
MM_2929	Hypothetical protein	3524542 _ 3524216	-0.18	0.51
MM_2930	Hypothetical protein	3526346 _ 3525156	-0.19	0.25
MM_2931	Hydrolase	3527826 _ 3526459	0.71	0.20
MM_2933	Conserved protein	3531138 _ 3529552	-0.30	0.78
MM_2937	Conserved protein	3535236 _ 3537854	-0.08	0.15
MM_2939	Serine O-acetyltransferase	3541776 _ 3541153	0.25	0.39
MM_2941	Hypothetical protein	3543138 _ 3543890	0.38	0.26
MM_2942	Conserved protein	3544170 _ 3544511	0.15	0.20
MM_2944	Conserved protein	3547122 _ 3546115	0.08	0.72
MM_2947	Conserved protein	3552999 _ 3553520	-0.56	0.21
MM_2949	Hypothetical protein	3555723 _ 3555325	-0.01	0.55
MM_2950	Conserved protein	3556106 _ 3555777	-0.04	0.61
MM_2951	Hypothetical protein	3557252 _ 3557713	0.16	0.72
MM_2952	Conserved protein	3560598 _ 3562049	0.02	0.44
MM_2954	Transcriptional regulator	3563668 _ 3563201	0.05	0.40
MM_2955	Hypothetical sensory transduction histidine kinase	3566295 _ 3563668	0.33	0.46

MM_2956	Conserved protein	3567192 _ 3566410	0.44	0.33
MM_2958	Conserved protein	3571114 _ 3570299	0.28	0.59
MM_2959	Hypothetical protein	3572325 _ 3571456	0.34	0.09
MM_2964	Dimethylamine permease MtbP	3575626 _ 3577227	1.90	0.87
MM_2965	Hypothetical sensory transduction histidine kinase	3577497 _ 3580013	0.07	0.36
MM_2967	Isoleucyl-tRNA synthetase	3581963 _ 3585136	-0.16	0.32
MM_2968	Conserved protein	3585218 _ 3585856	-0.21	0.30
MM_2972	Hypothetical protein	3588766 _ 3590139	0.71	0.25
MM_2973	Hypothetical protein	3591402 _ 3590194	-0.22	0.38
MM_2977	Hypothetical protein	3598236 _ 3596878	-0.19	0.21
MM_2978	Type I restriction-modification system specificity su	3599627 _ 3598248	0.03	0.91
MM_2979	Hypothetical protein	3600715 _ 3599627	-0.11	0.25
MM_2980	Hypothetical protein	3600936 _ 3600703	0.12	0.73
MM_2981	Type I restriction-modification system methylation su	3603077 _ 3600936	-0.26	0.44
MM_2984	Putative ferredoxin	3604706 _ 3604933	0.21	0.53
MM_2985	Transcriptional regulator, ArsR family	3606230 _ 3605457	-0.18	0.33
MM_2987	Hypothetical protein	3608670 _ 3606748	-0.03	0.53
MM_2988	Conserved protein	3611810 _ 3610896	0.50	0.47
MM_2993	Phosphoglycerate mutase	3617877 _ 3617155	-0.20	0.30
MM_2994	Conserved protein	3618840 _ 3618193	-0.24	0.61
MM_2996	Hypothetical protein	3621279 _ 3620422	-0.57	0.44
MM_2998	Transposase	3622587 _ 3621931	0.14	0.56
MM_3000	DNA repair helicase (RAD25/XPB family)	3623764 _ 3625185	-0.14	0.25
MM_3004	Conserved protein	3630411 _ 3629125	-0.01	0.62
MM_3005	Acetyltransferase	3631040 _ 3630591	-0.21	0.47
MM_3007	Probable transcriptional regulator	3632390 _ 3633265	0.50	0.20
MM_3009	Membrane metalloprotease	3636335 _ 3634518	-0.01	0.34
MM_3010	Periplasmic divalent cation tolerance protein	3637104 _ 3637406	-0.21	0.34
MM_3011	Hypothetical protein	3638676 _ 3637879	-0.34	0.36
MM_3012	Hypothetical protein	3640397 _ 3639225	0.10	0.62
MM_3013	ABC transporter, permease protein	3640923 _ 3641996	-0.36	0.35
MM_3014	Conserved protein	3642051 _ 3642980	-0.02	0.30
MM_3015	Hypothetical protein	3643027 _ 3644115	0.17	0.24
MM_3016	Cobalt transport ATP-binding protein CbiO	3646005 _ 3644236	-0.16	0.31
MM_3017	ABC transporter, permease protein	3646115 _ 3646975	0.07	0.46
MM_3018	Conserved protein	3648179 _ 3647079	-0.09	0.36
MM_3019	Conserved protein	3648920 _ 3650701	-0.16	0.40
MM_3020	Conserved protein	3650661 _ 3651656	0.04	0.30
MM_3021	ABC transporter, ATP-binding protein	3651663 _ 3652370	-0.18	0.39
MM_3022	Conserved protein	3654047 _ 3652458	0.12	0.54
MM_3023	Conserved protein	3655753 _ 3654059	0.19	0.44
MM_3024	Conserved protein	3657153 _ 3660197	-0.14	0.30
MM_3025	Hypothetical protein	3660658 _ 3661776	-0.38	0.61
MM_3027	Transposase	3665628 _ 3664018	-0.29	0.51
MM_3028	Deoxycytidylate deaminase	3666813 _ 3666298	0.23	0.23
MM_3029	Lactoylglutathione lyase	3667292 _ 3667681	0.28	0.48
MM_3030	Conserved protein	3668735 _ 3669280	0.33	0.59
MM_3032	Conserved protein	3670674 _ 3669952	0.40	0.18
MM_3034	Conserved protein	3671240 _ 3671677	-0.26	0.15
MM_3037	Transposase	3673894 _ 3674301	0.18	0.18
MM_3038	Transposase	3674305 _ 3675423	0.09	0.52
MM_3041	Hypothetical protein	3682374 _ 3677245	-0.39	0.48
MM_3042	Coenzyme F420 hydrogenase, beta su	3683615 _ 3682734	-0.26	0.62
MM_3043	Coenzyme F420 hydrogenase, gamma su	3684480 _ 3683620	-0.37	0.31
MM_3044	Coenzyme F420 hydrogenase, delta su	3684955 _ 3684470	-0.29	0.13
MM_3045	Coenzyme F420 hydrogenase, alpha su	3686319 _ 3684955	-0.16	0.37
MM_3052	Oxidoreductase (hypothetical)	3693205 _ 3695364	-0.13	0.40
MM_3053	CapK protein	3695423 _ 3696817	-0.05	0.30
MM_3057	Hypothetical protein	3699582 _ 3699815	0.21	0.27
MM_3059	Conserved protein	3701142 _ 3701927	0.30	0.26

MM_3060	Hypothetical protein	3702525 _ 3702007	-0.04	0.41
MM_3062	Hypothetical protein	3706237 _ 3705185	0.53	0.30
MM_3065	Hypothetical protein	3714686 _ 3712272	-0.19	0.16
MM_3066	Fumarate hydratase, alpha su	3715627 _ 3716517	0.02	0.44
MM_3067	Fumarate hydratase, beta su	3716520 _ 3717095	-0.04	0.63
MM_3068	Conserved protein	3718074 _ 3717193	0.09	0.31
MM_3074	RNase P RNA component	3729492 _ 3730010	-0.09	0.70
MM_3075	Hypothetical protein	3730047 _ 3730514	0.06	0.67
MM_3076	DNA topoisomerase I	3730798 _ 3733053	-0.26	0.49
MM_3077	Probable transcriptional regulator	3733774 _ 3734217	-0.13	0.33
MM_3078	mRNA 3'-end processing factor	3734775 _ 3736022	0.09	0.48
MM_3079	Hypothetical protein	3736283 _ 3736651	0.03	0.70
MM_3081	Serine/threonine protein kinases	3740490 _ 3739441	0.07	0.44
MM_3082	Hypothetical protein	3740773 _ 3741951	0.29	0.45
MM_3084	Protease I	3745073 _ 3744642	0.23	0.24
MM_3086	Nicotinate phosphoribosyltransferase	3746722 _ 3747939	0.10	0.25
MM_3087	Hypothetical protein	3749767 _ 3748529	-0.02	0.21
MM_3088	Carbonic anhydrase	3751409 _ 3750681	-0.01	0.40
MM_3089	Hypothetical protein	3751638 _ 3752333	0.01	0.43
MM_3093	Hypothetical protein	3757175 _ 3758059	0.16	0.45
MM_3094	Hypothetical protein	3758156 _ 3759349	0.16	0.30
MM_3098	ABC transporter, permease protein	3762879 _ 3764114	-0.19	0.27
MM_3099	Two component system histidine kinase	3767903 _ 3765783	0.06	0.53
MM_3100	Hypothetical protein	3768696 _ 3769412	-0.03	0.71
MM_3104	Hypothetical protein	3774022 _ 3773621	-0.01	0.50
MM_3105	Probable cytosine deaminase	3774175 _ 3774792	0.08	0.56
MM_3107	Hypothetical protein	3776604 _ 3776167	0.39	0.39
MM_3108	Hypothetical protein	3777826 _ 3776846	0.21	0.28
MM_3109	Glycogen phosphorylase	3778566 _ 3780242	0.15	0.68
MM_3110	Hypothetical protein	3781598 _ 3780420	-0.30	0.22
MM_3111	Hypothetical protein	3783501 _ 3782185	-0.16	0.41
MM_3113	Oligoendopeptidase F	3786412 _ 3784562	0.18	0.37
MM_3114	Conserved protein	3786788 _ 3786994	-0.05	0.60
MM_3115	Hypothetical protein	3787325 _ 3788632	-0.10	0.37
MM_3116	Hypothetical protein	3789455 _ 3788901	0.11	0.49
MM_3117	Transcriptional regulator, MarR family	3789530 _ 3789946	0.46	0.04
MM_3118	ATP-dependent protease La	3790130 _ 3792514	0.54	0.17
MM_3119	Iron-sulfur flavoprotein	3792833 _ 3793513	0.25	0.12
MM_3120	Hypothetical protein	3793988 _ 3793608	-0.32	0.67
MM_3122	Putative hydrolase	3795965 _ 3796756	-0.18	0.46
MM_3123	Hypothetical protein	3796868 _ 3797500	0.10	0.31
MM_3124	Hypothetical protein	3797989 _ 3798921	-0.17	0.32
MM_3125	Hypothetical protein	3799684 _ 3799097	0.07	0.07
MM_3126	Hypothetical protein	3799881 _ 3800408	-0.09	0.18
MM_3128	Hypothetical protein	3801199 _ 3801798	-0.02	0.45
MM_3130	Hypothetical protein	3803988 _ 3802648	-0.21	0.25
MM_3132	Hypothetical protein	3806374 _ 3805811	-0.37	0.33
MM_3134	Protease HTPX	3808379 _ 3807498	-0.36	0.27
MM_3135	ABC transporter, ATP-binding protein	3809135 _ 3809935	-0.36	0.36
MM_3136	ABC transporter, permease protein	3809935 _ 3810684	0.12	0.43
MM_3137	TRANSPORTER, LysE family	3810925 _ 3811548	0.18	0.49
MM_3139	Large-conductance mechanosensitive channel	3814017 _ 3813787	0.08	0.43
MM_3140	Flavodoxin	3814687 _ 3815214	0.11	0.08
MM_3142	Type I restriction-modification system specificity su	3818738 _ 3819931	-0.05	0.31
MM_3143	Hypothetical protein	3821545 _ 3820355	-0.12	0.19
MM_3145	Hypothetical protein	3822518 _ 3822297	0.29	0.48
MM_3147	Type I restriction-modification system methylation su	3825310 _ 3826821	0.03	0.52
MM_3148	Exodeoxyribonuclease III	3827741 _ 3826968	-0.10	0.29
MM_3149	Hypothetical protein	3828955 _ 3829314	0.54	0.46
MM_3150	Methyltransferase	3830177 _ 3831205	0.06	0.53

MM_3152	Hypothetical protein	3832420 _ 3832040	-0.08	0.92
MM_3154	Hypothetical protein	3834481 _ 3833957	0.25	0.55
MM_3157	Putative 6-aminohexanoate-dimer hydrolase	3838379 _ 3836817	-0.13	0.45
MM_3162	Hypothetical protein	3844614 _ 3844922	0.00	0.27
MM_3164	Conserved protein	3845853 _ 3846719	-0.18	0.36
MM_3165	Hypothetical protein	3848698 _ 3848964	-0.18	0.06
MM_3166	Hypothetical protein	3849381 _ 3849001	-0.29	0.32
MM_3168	Conserved protein	3853325 _ 3852075	-0.05	0.23
MM_3170	Transcriptional regulator, MerR family	3854377 _ 3855228	0.20	0.01
MM_3171	Hypothetical protein	3856425 _ 3857708	0.13	0.38
MM_3172	Putative methyltransferase	3858776 _ 3857922	-0.13	0.30
MM_3173	Transcriptional regulator, ArsR family	3859321 _ 3859022	-0.11	0.43
MM_3177	Hypothetical protein	3861352 _ 3862167	0.58	0.27
MM_3178	Hypothetical protein	3863965 _ 3862250	0.22	0.33
MM_3179	Transcriptional regulator	3864590 _ 3865147	0.24	0.33
MM_3180	Putative acetyl-CoA synthetase	3865303 _ 3866982	0.02	0.27
MM_3181	Ketoisovalerate oxidoreductase su	3867269 _ 3867514	-0.10	0.53
MM_3182	Ketoisovalerate oxidoreductase su	3867537 _ 3868589	-0.09	0.13
MM_3183	Ketoisovalerate oxidoreductase su	3868595 _ 3870037	-0.10	0.58
MM_3185	Ornithine decarboxylase	3873090 _ 3871921	-0.26	0.48
MM_3187	Flavodoxin	3876572 _ 3875379	0.35	0.41
MM_3189	Potassium channel protein	3878825 _ 3879661	0.00	0.53
MM_3190	Hypothetical protein	3879791 _ 3880681	-0.17	0.19
MM_3191	Hypothetical protein	3880799 _ 3881170	-0.36	0.49
MM_3193	Hypothetical membrane associated protein	3882138 _ 3881929	0.29	0.25
MM_3195	Transcriptional regulator, MarR family	3884185 _ 3883721	-0.04	0.72
MM_3198	Hypothetical protein	3887391 _ 3886000	-0.04	0.47
MM_3199	Transporter	3887532 _ 3888464	-0.01	0.53
MM_3201	Conserved protein	3889085 _ 3889801	0.38	0.47
MM_3202	Conserved protein	3892393 _ 3889814	-0.01	0.68
MM_3205	Two component system histidine kinase	3900532 _ 3897857	0.06	0.32
MM_3208	Transcriptional regulator, ArsR family	3904763 _ 3905167	0.01	0.50
MM_3209	Transporter	3905363 _ 3906280	-0.12	0.59
MM_3213	Prismane protein	3910046 _ 3911665	0.02	0.22
MM_3214	Ferredoxin	3912667 _ 3911822	-0.04	0.42
MM_3215	Conserved protein	3913518 _ 3913850	0.05	0.35
MM_3216	Conserved protein	3915108 _ 3914176	-0.28	0.82
MM_3217	Conserved protein	3916279 _ 3915956	-0.23	0.04
MM_3219	Conserved protein	3918109 _ 3917204	0.21	0.54
MM_3220	Transporter	3918314 _ 3919756	0.16	0.39
MM_3223	ABC transporter, ATP-binding protein	3922057 _ 3922836	0.17	0.63
MM_3225	Putative ketoreductase	3923854 _ 3924561	-0.02	0.10
MM_3230	Phosphate permease	3927396 _ 3928418	-0.34	0.66
MM_3231	Conserved protein	3928421 _ 3929107	0.15	0.35
MM_3232	Hypothetical protein	3929413 _ 3929646	-0.17	0.30
MM_3233	HTH DNA-binding protein	3930262 _ 3929975	0.16	1.00
MM_3234	Hydroxyethylthiazole kinase	3930505 _ 3931287	-0.10	0.34
MM_3235	Thiamin-phosphate pyrophosphorylase	3931531 _ 3932250	-0.27	0.50
MM_3238	Conserved protein	3933749 _ 3934312	-0.04	0.27
MM_3240	Probable transcriptional regulator	3935262 _ 3936632	0.06	0.19
MM_3244	Hypothetical protein	3942306 _ 3939700	-0.33	0.86
MM_3248	Hypothetical protein	3948764 _ 3948090	-0.15	0.38
MM_3249	Hypothetical protein	3950274 _ 3949060	-0.06	1.04
MM_3252	Hypothetical protein	3952047 _ 3952376	0.01	0.52
MM_3255	Hypothetical protein	3957188 _ 3957526	0.16	0.62
MM_3256	Hypothetical protein	3958282 _ 3959499	-0.06	0.70
MM_3258	Hypothetical protein	3960550 _ 3961593	0.31	0.37
MM_3259	Conserved protein	3961597 _ 3962520	0.25	0.36
MM_3260	Hypothetical protein	3963127 _ 3962615	0.37	0.54
MM_3262	Branched-chain amino acid transport protein	3964659 _ 3965390	-0.34	0.47

MM_3264	F420 hydrogenase/dehydrogenase, beta su	3965865 _ 3966752	-0.43	0.32
MM_3268	NADPH-flavin oxidoreductase	3972364 _ 3971843	0.20	0.63
MM_3270	Ferredoxin-thioredoxin reductase, catalytic chain	3974513 _ 3973971	0.07	0.34
MM_3271	Glutaredoxin	3974828 _ 3974529	0.08	0.11
MM_3272	Putative transport protein	3976509 _ 3975025	-0.14	0.56
MM_3274	Hypothetical protein	3978243 _ 3979364	-0.14	0.43
MM_3275	Conserved protein	3979486 _ 3980793	-0.41	0.45
MM_3276	Hypothetical protein	3982543 _ 3980870	0.12	0.30
MM_3277	Protease I	3983289 _ 3982693	-0.11	0.25
MM_3278	Hypothetical Membrane Spanning protein	3984487 _ 3983429	0.12	0.19
MM_3280	Conserved protein	3989477 _ 3986982	0.03	0.10
MM_3281	SAM-dependent methyltransferases	3991158 _ 3990313	-0.30	0.42
MM_3282	Probable ATP-dependent helicase	3993359 _ 3991422	-0.30	0.26
MM_3283	Putative inorganic pyrophosphatase	3993610 _ 3994032	0.08	0.17
MM_3284	Transcriptional regulator	3994312 _ 3995448	0.21	0.64
MM_3286	Hypothetical protein	3996944 _ 3997312	0.00	0.36
MM_3287	Hypothetical protein	3998626 _ 3998048	0.08	0.68
MM_3288	Conserved protein	3999805 _ 4000389	-0.03	0.49
MM_3289	Excinuclease ABC, su B	4002519 _ 4000510	0.45	0.45
MM_3290	Excinuclease ABC, su C	4004173 _ 4002605	-0.10	0.48
MM_3291	Excinuclease ABC, su A	4007352 _ 4004368	-0.16	0.39
MM_3293	Iron-sulfur cluster-binding protein	4008945 _ 4009805	0.36	0.44
MM_3295	Hypothetical sensory transduction histidine kinase	4013955 _ 4011187	0.53	0.32
MM_3296	Hypothetical protein	4014377 _ 4014565	0.03	0.36
MM_3300	Phosphoadenosine phosphosulfate reductase	4017939 _ 4020152	-0.14	0.36
MM_3303	Hypothetical protein	4022097 _ 4023194	0.09	0.13
MM_3304	Threonyl-tRNA synthetase	4023468 _ 4025369	-0.24	0.46
MM_3305	Putative DNA or RNA helicase of superfamily II	4025916 _ 4028096	0.00	0.49
MM_3306	Conserved protein	4028835 _ 4029647	-0.35	0.30
MM_3307	Conserved protein	4030370 _ 4029843	0.39	0.30
MM_3308	Conserved protein	4030662 _ 4031378	-0.16	0.56
MM_3311	Acetyltransferase	4033228 _ 4033857	0.17	0.98
MM_3312	5-Methylcytosine-specific restriction enzyme A	4034742 _ 4033972	0.46	0.21
MM_3314	N-5'-phosphoribosyl)anthranilate isomerase	4036559 _ 4035801	-0.22	0.17
MM_3315	Hypothetical protein	4036744 _ 4037124	-0.27	0.35
MM_3318	Conserved protein	4039791 _ 4043282	-0.04	0.49
MM_3319	Transport protein	4044831 _ 4043521	0.33	0.58
MM_3324	Oligopeptide transporter, ATP-binding protein	4050309 _ 4049425	-0.12	0.40
MM_3325	Permease, Na+/H+-dicarboxylate symporter	4051516 _ 4052835	0.35	0.20
MM_3326	Aldehyde ferredoxin oxidoreductase	4054828 _ 4053065	0.48	0.58
MM_3328	Conserved protein	4057061 _ 4056360	-0.01	0.28
MM_3331	Conserved protein	4059828 _ 4061279	0.34	0.21
MM_3333	Hypothetical protein	4062395 _ 4061961	0.31	0.48
MM_3335	Monomethylamine:corrinoid methyltransferase MtmB2	4063387 _ 4063989	1.61	0.65
MM_3336	Monomethylamine:corrinoid methyltransferase MtmB2	4064041 _ 4064760	0.27	0.51
MM_3338	Hypothetical protein	4065728 _ 4066552	0.09	0.42
MM_3339	Hypothetical protein	4066716 _ 4067546	0.12	0.56
MM_3342	Conserved protein	4070261 _ 4071511	-0.33	0.27
MM_3343	Transposase	4071750 _ 4072241	0.11	0.67
MM_3345	Hypothetical protein	4073159 _ 4073482	-0.45	0.44
MM_3346	Hypothetical protein	4074683 _ 4074204	0.48	0.35
MM_3347	Conserved protein	4074963 _ 4075409	0.43	0.39
MM_3350	Hypothetical protein	4077845 _ 4077282	0.09	0.57
MM_3353	Conserved protein	4081273 _ 4080455	0.14	0.32
MM_3355	Conserved protein	4082623 _ 4081679	-0.29	0.40
MM_3357	Hypothetical protein	4086488 _ 4085379	-0.20	0.53
MM_3358	Hypothetical protein	4087769 _ 4086672	0.10	0.22
MM_3361	Hypothetical protein	4089787 _ 4090134	0.02	0.66
MM_3370	Hypothetical protein	4095157 _ 4094735	-0.29	0.51
MM_3371	Conserved protein	4096173 _ 4095400	-0.21	0.10

Tab. 10.4: List of the microarray data derived from experiments performed with *M. mazei* cultures exposed to 100 µM KH_2AsO_4 (As(V)) or without metalloid.
Data were derived from one experiment, including one dye swap reaction, neglecting contrary results from the dye swap reaction. Data already listed in the result section are excluded.

Orf No.	Annotated geneproduct	mean expression ratios 100 µM As(V) vs. control (log2)
MM_0001	Dipeptide ABC transporter, binding protein	-0.11
MM_0002	Dipeptide ABC transporter, binding protein	-0.43
MM_0004	Dipeptide ABC transporter, permease protein	-0.21
MM_0005	Dipeptide ABC transporter, permease protein	-0.43
MM_0007	Dipeptide ABC transporter, ATP-binding protein	-0.69
MM_0010	Hypothetical protein	0.21
MM_0017	Ubiquinone/menaquinone biosynthesis methyltransferase	0.58
MM_0021	Conserved protein	0.64
MM_0024	Hypothetical protein	0.36
MM_0030	Tetratrico peptide repeat protein	1.00
MM_0037	Argininosuccinate synthase	-0.61
MM_0038	Carbamoyl-phosphate synthase large chain	-0.76
MM_0039	Carbamoyl-phosphate synthase small chain	-0.80
MM_0040	Glycine betaine transporter, ATP-binding protein (OtaA)	0.58
MM_0043	Conserved protein	0.15
MM_0046	Hypothetical protein	0.77
MM_0047	Acetylornithine aminotransferase	-1.37
MM_0050	Glutamyl-tRNA (Gln) amidotransferase	0.19
MM_0057	Tungsten formylmethanofuran dehydrogenase, su F	0.13
MM_0058	Tungsten formylmethanofuran dehydrogenase, su G	0.08
MM_0059	Tungsten formylmethanofuran dehydrogenase su B	0.19
MM_0062	Putative methyltransferase	0.21
MM_0063	Cell surface glycoprotein (s-layer protein)	0.23
MM_0067	Hypothetical protein	-0.09
MM_0072	Thermosome beta-su	0.55
MM_0077	Hypothetical protein	-0.92
MM_0080	Hypothetical protein	0.21
MM_0090	Conserved protein	0.86
MM_0092	Conserved protein	0.33
MM_0093	Cobyric acid synthase CbiP	-0.29
MM_0095	Conserved protein	-0.52
MM_0096	Transposase	-0.06
MM_0097	Ornithine cyclodeaminase	0.90
MM_0098	Conserved protein	0.39
MM_0101	Conserved protein	-0.43
MM_0102	Serine/threonine protein phosphatase	0.36
MM_0107	Hypothetical protein	-0.69
MM_0113	Conserved protein	0.07

MM_0114	Conserved protein	-0.72
MM_0118	CTP synthase	-1.06
MM_0119	Hypothetical protein	-0.02
MM_0120	Conserved protein	-0.42
MM_0123	Universal stress protein	-0.58
MM_0125	Universal stress protein	-0.43
MM_0128	ATP-dependent protease La	0.05
MM_0129	Conserved protein	-0.22
MM_0130	Conserved protein	0.30
MM_0131	Conserved protein	1.02
MM_0133	Threonine synthase	-3.19
MM_0134	Sulfopyruvate decarboxylase alpha chain	-3.12
MM_0136	Zn-dependent hydrolase	0.18
MM_0137	Conserved protein	1.47
MM_0138	Conserved protein	0.13
MM_0142	Orotate phosphoribosyltransferase	-0.82
MM_0144	Phosphoribosylamine--glycine ligase	-0.19
MM_0145	Ornithine carbamoyltransferase	-0.26
MM_0146	Transcriptional regulator	-0.32
MM_0150	putative nucleoside-diphosphate-sugar epimerase	-0.37
MM_0156	Type III restriction enzyme	0.43
MM_0159	Hypothetical protein	0.10
MM_0174	Methanol corrinoid protein MtaC3	1.05
MM_0182	Thiamine biosynthesis protein	-0.95
MM_0184	SSU ribosomal protein S3AE	-0.84
MM_0187	Nucleotidyltransferase	1.20
MM_0188	Putative nucleotidyltransferase	0.87
MM_0195	Conserved protein	-0.12
MM_0200	Conserved protein	0.07
MM_0201	Conserved protein	0.17
MM_0203	Hypothetical protein	-0.29
MM_0211	Cysteine proteinase	-0.32
MM_0212	Conserved protein	-0.42
MM_0213	Putative single-stranded-DNA-specific exonuclease	-0.13
MM_0218	Conserved protein	-0.46
MM_0219	Conserved protein	-0.06
MM_0230	Sodium-calcium exchanger	-0.94
MM_0235	Conserved protein	0.25
MM_0237	Conserved protein	0.97
MM_0239	Conserved protein	1.86
MM_0240	Ferredoxin	-0.52
MM_0241	Potential ferredoxin oxidoreductase	-0.50

MM_0242	Glycerol-3-phosphate cytidylyltransferase	-0.68
MM_0244	Riboflavin synthase, su beta	-0.65
MM_0247	Putative inosine monophosphate dehydrogenase	0.60
MM_0262	Conserved protein	-0.04
MM_0263	Conserved protein	-0.05
MM_0264	Conserved protein	-0.55
MM_0268	Conserved protein	0.47
MM_0275	Hypothetical protein	-0.62
MM_0278	DNA-cytosine methyltransferase	-0.63
MM_0279	Membrane alanine aminopeptidase	-0.30
MM_0282	Threonine synthase	-0.28
MM_0284	Amidohydrolase (putative)	-0.11
MM_0285	Conserved protein	-0.07
MM_0286	Hypothetical protein	-1.10
MM_0287	Deoxyhypusine synthase	-0.45
MM_0288	Transcriptional regulator, ArsR family	-0.36
MM_0290	Hypothetical protein	-0.25
MM_0293	Conserved protein	0.69
MM_0299	Glucose-1-phosphate thymidylyltransferase	-0.48
MM_0300	Glucosamine-fructose-6-phosphate aminotransferase [isomerizing]	-0.48
MM_0301	Phosphoglucomutase/phosphomannomutase	-0.88
MM_0302	Glucose-1-phosphate thymidylyltransferase	-0.91
MM_0304	AAA family ATPase	0.66
MM_0306	Metal-dependent hydrolases	-0.22
MM_0308	Uroporphyrinogen-III synthase	-0.59
MM_0309	Metallo cofactor biosynthesis protein moaA/ nifB /pqqE family	-0.37
MM_0311	Conserved protein	-0.17
MM_0314	Valyl-tRNA synthetase	-0.33
MM_0329	Protein-glutamate methylesterase CheB	-0.17
MM_0334	Hypothetical protein	0.25
MM_0335	3-Hydroxy-3-methylglutaryl-coenzyme A reductase	-0.07
MM_0338	Putative snRNP Sm-like protein	-0.09
MM_0339	Putative snRNP Sm-like protein	-0.89
MM_0344	Probable cation efflux pump	-0.50
MM_0345	Transcriptional regulator	-0.05
MM_0348	Putative cation efflux pump	-0.14
MM_0350	Putative molybdopterin biosynthesis protein	-2.04
MM_0351	Conserved protein	-0.17
MM_0352	Hypothetical protein	-0.16
MM_0357	Glutamate dehydrogenase	0.02
MM_0360	Sulfite reductase, assimilatory-type	0.35

MM_0361	Transcriptional regulator, MarR family	-0.67
MM_0362	Sulfite reductase, assimilatory-type	-0.08
MM_0363	Hypothetical protein	-0.91
MM_0364	Hypothetical protein	-0.92
MM_0365	Conserved protein	-1.06
MM_0371	Conserved protein	0.25
MM_0374	Conserved protein	-0.51
MM_0375	Conserved protein	0.00
MM_0377	Integral membrane protein	1.19
MM_0378	Sugar-phosphate nucleotidyltransferase	0.12
MM_0381	Peptidyl-prolyl cis-trans isomerase	-0.36
MM_0382	Peptidyl-prolyl cis-trans isomerase	-0.52
MM_0386	Hypothetical protein	1.01
MM_0387	Heterodisulfate reductase, su HdrA	-2.37
MM_0388	Heterodisulfate reductase, su HdrC	-3.14
MM_0389	Heterodisulfate reductase, su HdrB	-2.36
MM_0394	Conserved protein	0.89
MM_0395	Surface layer protein (putative)	0.29
MM_0396	Transposase	-0.17
MM_0398	Conserved protein	0.72
MM_0402	Conserved protein	1.44
MM_0405	Surface layer protein (putative)	-0.04
MM_0406	Conserved protein	0.59
MM_0407	Hypothetical protein similar to C-terminal domain of HdrB	-0.34
MM_0408	Hypothetical protein	0.41
MM_0409	3-Isopropylmalate dehydratase	-1.44
MM_0419	Pyruvate synthase, su beta	-0.12
MM_0420	Pyruvate synthase, su alpha	-0.50
MM_0424	Histidinol dehydrogenase	-0.54
MM_0428	Conserved protein	-0.35
MM_0429	Type I restriction-modification system methylation su	0.31
MM_0430	Type I restriction-modification system specificity su	-0.03
MM_0436	Thioredoxin	0.10
MM_0437	Hypothetical protein	-0.26
MM_0438	Methylenetetrahydrofolate reductase	1.28
MM_0439	Zinc finger protein	0.01
MM_0443	Phosphoribosylglycinamide formyltransferase	-0.15
MM_0447	Cell division control protein (AAA family ATPase)	-0.20
MM_0449	SAM-dependent methyltransferases	0.08
MM_0453	Conserved protein	-0.18
MM_0454	Hypothetical protein	-0.37
MM_0457	Recombination/repair protein RadA	0.50

MM_0459	Conserved protein	0.50
MM_0460	Probable endonuclease IV	0.17
MM_0463	Cobalt ABC transporter, permease protein CbiQ	0.00
MM_0469	Hypothetical protein	-0.28
MM_0473	Glucokinase	-0.23
MM_0475	Putative inosine-5'-monophosphate dehydrogenase	0.03
MM_0476	N-acetyl-gamma-glutamyl-phosphate reductase	-0.08
MM_0479	Conserved protein	0.79
MM_0484	Hypothetical protein	0.44
MM_0485	Phosphoglycerate kinase	0.36
MM_0486	DNA polymerase	-0.75
MM_0487	Heat shock protein	-0.58
MM_0493	Acetyl-CoA synthetase, beta su	1.19
MM_0495	Acetate kinase	0.56
MM_0496	Phosphate acetyltransferase	1.42
MM_0497	Iron-sulfur flavoprotein (Isf)	-0.10
MM_0498	Putative chloride channel protein	-0.98
MM_0511	Conserved protein	-0.42
MM_0514	Nitrogenase iron protein	-0.26
MM_0515	Conserved protein	-0.09
MM_0517	Conserved protein	0.97
MM_0518	Hypothetical sensory transduction histidine kinase	0.49
MM_0531	Conserved protein	0.36
MM_0534	Conserved protein	0.44
MM_0536	Hypothetical protein	0.18
MM_0537	Hypothetical protein	0.53
MM_0542	Hypothetical protein	-0.20
MM_0552	Hypothetical protein	-0.37
MM_0555	Hypothetical protein	0.42
MM_0565	Transcriptional regulator	0.41
MM_0567	Acetyltransferase	0.15
MM_0577	Phosphate transport ATP-binding protein	-0.18
MM_0578	Phosphate-binding protein	-0.83
MM_0584	Conserved protein	0.56
MM_0585	Conserved protein	0.44
MM_0589	Conserved protein	-0.33
MM_0590	Glycerol-1-phosphate dehydrogenase (putative)	0.68
MM_0591	Conserved membrane protein	0.19
MM_0593	Conserved protein	-0.86
MM_0594	Protein translation initiation factor 2 su gamma (IF-2g)	-0.26
MM_0595	Conserved protein	-1.05
MM_0596	DNA-directed RNA polymerase su E'	-0.48

MM_0598	Hypothetical protein	-1.06
MM_0602	O-sialoglycoprotein endopeptidase	-0.33
MM_0608	Probable radical-forming protein	0.22
MM_0610	Acetyltransferases	-0.17
MM_0614	Long-chain-fatty-acid--CoA ligase	-0.32
MM_0618	Undecaprenyl pyrophosphate synthetase	-0.17
MM_0619	Conserved protein	-0.51
MM_0620	Conserved protein	-0.59
MM_0621	Hypothetical protein	-0.56
MM_0626	Nicotinamide-nucleotide adenylyltransferase	-0.37
MM_0627	F420H2 dehydrogenase, su FpoF	-0.20
MM_0628	Coenzyme F420-dependent N5,N10-methylene tetrahydromethanopterin reductase	-0.29
MM_0629	Zinc finger protein	-0.18
MM_0631	Hypothetical protein similar to COX locus protein	0.04
MM_0632	Desulfoferrodoxin	-0.24
MM_0633	Cytochrome c	-0.02
MM_0634	Iron-sulfur flavoprotein	-0.33
MM_0635	Flavoprotein	-0.41
MM_0636	Conserved protein	-0.04
MM_0637	Putative flavodoxin	-0.44
MM_0642	Isocitrate dehydrogenase [NADP]	-0.03
MM_0644	Conserved protein	-0.38
MM_0645	3-Isopropylmalate dehydratase	-0.22
MM_0646	Oligosaccharyl transferase	0.06
MM_0649	Glycosyl transferase	1.14
MM_0650	Mannosyltransferase	-0.19
MM_0651	Glycosyltransferase involved in cell wall biogenesis	-0.25
MM_0652	Glycosyltransferase involved in cell wall biogenesis	-0.45
MM_0656	Polysaccharide ABC transporter, ATP-binding protein	-0.64
MM_0658	GDP-fucose synthetase	-0.90
MM_0659	GDP-mannose 4,6 dehydratase	-0.34
MM_0660	Mannose-6-phosphate isomerase/ mannose-1-phosphate guanylyl transferase	-0.43
MM_0662	Transcriptional regulator, ArsR family	0.09
MM_0663	Probable dihydroorotate dehydrogenase electron transfer su	-0.95
MM_0664	Glutamate synthase [NADPH]	-0.51
MM_0666	Putative nickel-responsive regulator NikR	-0.05
MM_0668	Ketol-acid reductoisomerase	-0.31
MM_0669	Acetolactate synthase small su	0.42
MM_0671	2-Isopropylmalate synthase	0.45
MM_0672	Hypothetical protein	-0.21
MM_0673	Conserved protein	0.14

MM_0674	Prefoldin beta su	0.75
MM_0675	Conserved protein	-0.04
MM_0676	Coenzyme F390 synthetase/phenylacetyl-CoA ligase	0.27
MM_0677	Acetolactate synthase small su	-0.22
MM_0678	Conserved protein	-0.06
MM_0679	Conserved protein	-0.06
MM_0680	Conserved protein	0.74
MM_0687	Nickel-insertion protein CooH	0.33
MM_0690	Conserved protein	-0.23
MM_0692	Transcription initiation factor IIE, alpha su	-0.53
MM_0693	Conserved protein	-1.29
MM_0694	Proteasome, beta su	-0.84
MM_0695	Cleavage and polyadenylation specificity factor, 100 kD su	-0.86
MM_0697	Cell division protein	0.05
MM_0698	Dihydropteroate synthase	0.30
MM_0700	Vacuolar-type H+-pyrophosphatase	-0.14
MM_0701	Vacuolar-type H+-pyrophosphatase	0.20
MM_0704	Conserved protein	-0.43
MM_0705	Conserved protein	0.35
MM_0706	ZPR1-related zinc finger protein	0.06
MM_0707	Prolyl-tRNA synthetase	-0.43
MM_0708	Conserved protein	-0.96
MM_0709	Conserved protein	-1.07
MM_0710	Hypothetical protein	0.09
MM_0712	Hypothetical protein	0.74
MM_0715	Pyruvate kinase	-0.51
MM_0716	Hypothetical protein	0.37
MM_0717	Hypothetical protein	0.07
MM_0718	Oxidoreductase (flavoprotein)	0.32
MM_0739	Transposase	-0.14
MM_0745	Conserved protein	0.54
MM_0747	ATP-dependent RNA helicase	-1.43
MM_0753	Conserved protein	-0.42
MM_0754	Conserved protein	-0.81
MM_0755	Conserved protein	-1.51
MM_0756	6-Pyruvoyltetrahydropterin synthase	-1.16
MM_0757	NifB protein	-0.16
MM_0762	Conserved protein	-0.21
MM_0764	Conserved protein	-0.37
MM_0765	Conserved protein	0.46
MM_0766	Transposase	1.11
MM_0767	Conserved protein	0.44

MM_0768	Glycosyl transferase	-0.67
MM_0772	Conserved protein	-0.93
MM_0774	Conserved protein	0.52
MM_0775	Conserved protein	0.05
MM_0778	A1AO H+ ATPASE, su D	-0.20
MM_0779	A1AO H+ ATPASE, su B	-0.36
MM_0780	A1AO H+ ATPASE, su A	-0.11
MM_0781	A1AO H+ ATPASE, su F	-0.86
MM_0782	A1AO H+ ATPASE, su C	-0.39
MM_0783	A1AO H+ ATPASE, su E	-0.68
MM_0784	A1AO H+ ATPASE, su K	0.27
MM_0785	A1AO H+ ATPASE, su I	0.46
MM_0786	A1AO H+ ATPASE, su H	0.50
MM_0788	Metallo cofactor biosynthesis protein moaA/ nifB /pqqE family	-0.22
MM_0789	Geranyltranstransferase/Farnesyltranstransferase/ Hexaprenyl diphosphate synthase	0.24
MM_0790	Hypothetical protein	0.17
MM_0798	26S Proteasome regulatory su RPT2/S4	-0.40
MM_0799	Hypothetical protein	0.60
MM_0801	Adenylosuccinate synthetase	-1.07
MM_0802	SSU ribosomal protein S19E	-0.62
MM_0803	Double-stranded DNA-binding protein	-1.01
MM_0804	Conserved hypothetical protein	-0.79
MM_0805	LSU ribosomal protein L39E	-1.07
MM_0807	Protein translation initiation factor 6 (IF-6)	-1.16
MM_0809	Prefoldin, alpha su	-0.95
MM_0810	Signal recognition particle, su Ffh/SRP54	0.16
MM_0814	Probable peroxiredoxin	-0.18
MM_0815	Transposase (N-terminal domain)	0.22
MM_0819	Gamma-glutamyl phosphate reductase	0.06
MM_0820	Conserved protein	0.03
MM_0823	Conserved protein	0.03
MM_0824	Universal stress protein	-0.24
MM_0825	Conserved protein	-0.43
MM_0827	Conserved protein	-0.58
MM_0829	Conserved protein	0.08
MM_0832	Conserved protein	-0.07
MM_0834	Na+/H+ antiporter	0.27
MM_0837	Conserved protein	0.44
MM_0841	Transcriptional regulator, ArsR family	-0.05
MM_0842	Conserved protein	-0.17
MM_0849	Bacterioferritin comigratory protein	0.56

MM_0851	Conserved protein	0.20
MM_0853	Conserved protein	-0.36
MM_0855	Phosphoribosylamidoimidazole-succinocarboxamide synthase	-0.80
MM_0858	Protein translation initiation factor 1 (IF-1)	0.75
MM_0859	Conserved protein	0.33
MM_0860	Phosphoribosylformylglycinamidine synthase	-0.38
MM_0861	Alpha-amylase	0.02
MM_0862	Alpha-amylase	0.37
MM_0863	Conserved protein	-0.16
MM_0865	Seryl-tRNA synthetase	-1.32
MM_0866	Periplasmic serine protease	-0.01
MM_0869	Conserved protein	0.12
MM_0870	Beta-ketoacyl synthase/ thiolase	-0.11
MM_0871	Hydroxymethylglutaryl-CoA synthase	-0.06
MM_0872	Putative transcriptional regulator	0.33
MM_0874	Conserved protein	0.22
MM_0876	Hypothetical protein	0.04
MM_0880	Conserved protein	-0.95
MM_0881	Transposase	-0.14
MM_0882	Conserved protein	-0.17
MM_0884	Conserved protein	0.16
MM_0887	Cobalt transport ATP-binding protein CbiO	0.34
MM_0888	Cobalt ABC transporter, permease protein CbiQ	-0.32
MM_0889	Hypothetical protein	-0.53
MM_0892	Hypothetical protein	-0.04
MM_0893	Cobalamin biosynthesis protein CbiM	0.55
MM_0894	Hypothetical protein	0.20
MM_0898	Bifunctional purine biosynthesis protein PurH	-0.75
MM_0899	Conserved protein	0.63
MM_0901	Cation-transporting ATPase	0.48
MM_0902	Putative methyltransferase	0.23
MM_0905	Conserved protein	0.21
MM_0906	Flap endonuclease-1 (RAD27/FEN1 family)	0.19
MM_0907	Hypothetical protein	0.40
MM_0908	Conserved protein	0.65
MM_0909	Conserved protein	0.69
MM_0910	Potassium/copper-transporting ATPase	0.40
MM_0911	ABC transporter, ATP-binding protein	-0.42
MM_0912	Conserved protein	-0.07
MM_0915	Conserved protein	-0.28
MM_0916	Conserved protein	-0.34
MM_0917	Conserved protein	-0.55

MM_0918	4-Carboxymuconolactone decarboxylase	-0.10
MM_0920	MutT related protein	-0.47
MM_0922	Protein iranslation initiation factor 5A (IF-5A)	-0.50
MM_0924	Conserved protein	0.51
MM_0933	Glutathione-regulated potassium-efflux system protein	0.28
MM_0936	Conserved protein	-0.54
MM_0941	Adenylosuccinate lyase	-0.11
MM_0942	Hypothetical protein	-0.49
MM_0943	Geranylgeranylglyceryl diphosphate synthase	-0.24
MM_0944	Methyl coenzyme M reductase, component A2 homolog	0.06
MM_0950	ABC transporter, ATP-binding protein	-0.07
MM_0961	Putative small heat shock protein	-0.38
MM_0962	Conserved protein	0.16
MM_0963	Integral Membrane protein	0.81
MM_0964	Glutamine synthetase	-0.39
MM_0966	Glutamate synthase, large chain	-1.51
MM_0967	Glutamate synthase, large chain	-2.14
MM_0969	F420 hydrogenase/dehydrogenase, beta su	0.23
MM_0970	Hypothetical protein	0.06
MM_0971	Hypothetical protein	0.84
MM_0974	Xaa-Pro aminopeptidase	0.19
MM_0975	Conserved protein	-0.10
MM_0976	Protease (putative)	-0.03
MM_0979	Heterodisulfate reductase, su HdrB	0.33
MM_0980	Heterodisulfate reductase, su HdrB	0.18
MM_0981	Iron-sulfur binding protein	-0.51
MM_0982	Transposase (N-terminal domain)	0.44
MM_0983	Conserved protein	-0.75
MM_0985	Conserved protein	-1.08
MM_0989	Conserved protein	-0.19
MM_0990	Nucleotide-binding protein	-0.60
MM_0991	Thiol-disulfide isomerase/thioredoxin	-0.23
MM_0994	Precorrin-8X methylmutase	-0.16
MM_0996	Cobalamin biosynthesis protein CbiG	-0.77
MM_0999	Precorrin-2 C20-methyltransferase	-0.72
MM_1001	Hypothetical protein	0.57
MM_1002	2-Isopropylmalate synthase	0.06
MM_1003	Isocitrate dehydrogenase [NADP]	-1.20
MM_1004	Putative molybdenum cofactor biosynthesis protein	-0.52
MM_1005	HTH DNA-binding protein	-0.91
MM_1006	26S Proteasome regulatory su RPT2/S5	0.37
MM_1007	Conserved protein	0.14

MM_1008	Cell division protein	0.41
MM_1009	Protein translocase, su SecE	-0.21
MM_1010	Putative transcription antitermination protein nusG	-0.64
MM_1012	LSU ribosomal protein L1P	-1.11
MM_1013	LSU ribosomal protein L10P	-1.14
MM_1014	LSU ribosomal protein L12AE	-0.77
MM_1015	Molybdenum cofactor biosynthesis enzyme (Fe-S oxidoreductase family)	0.37
MM_1017	Conserved protein	1.01
MM_1019	Iron-sulfur cluster-binding protein	-0.19
MM_1024	Conserved protein	-0.48
MM_1025	Thiamine biosynthesis protein	-1.16
MM_1026	Dolichol-phosphate mannosyltransferase	-0.62
MM_1027	Transcription initiation factor TFIIB	0.39
MM_1028	Transcription initiation factor TFIIB	0.06
MM_1030	Conserved protein	0.88
MM_1031	Chromosome partition protein	0.09
MM_1032	Conserved protein	0.93
MM_1033	Ferrous iron transport protein B	-0.19
MM_1034	Transcriptional repressor	-0.32
MM_1035	Amino-acid acetyltransferase	-0.13
MM_1036	BioY protein	0.32
MM_1037	Cobalt transport ATP-binding protein CbiO	-0.07
MM_1042	Transcriptional regulator	1.44
MM_1045	Branched-chain amino acid aminotransferase	-1.31
MM_1047	GTP-binding protein	0.58
MM_1048	D-alanine-D-alanine ligase related protein	-0.86
MM_1051	Chromosomal protein MC1	0.55
MM_1053	Putative Sensory protein	0.02
MM_1056	Putative ATP-dependent Na+ efflux pump	-0.01
MM_1057	ABC transporter, ATP-binding protein	0.22
MM_1059	Formate hydrogenlyase su 3	-0.40
MM_1060	Formate hydrogenlyase su 4	-0.91
MM_1063	Formate hydrogenlyase su 5 precursor	-1.07
MM_1064	Formate hydrogenlyase su 7	1.36
MM_1067	Conserved protein	0.15
MM_1071	Conserved protein	-0.97
MM_1072	Conserved protein	-1.01
MM_1073	Methanol corrinoid protein MtaC2	2.03
MM_1075	Putative regulatory protein	1.65
MM_1076	Cation efflux protein	1.51
MM_1077	Conserved protein	0.06
MM_1078	Conserved protein	0.26

MM_1081	Conserved protein	0.59
MM_1082	GTP-binding protein	-0.31
MM_1083	Undecaprenyl pyrophosphate synthetase	-0.37
MM_1085	Conserved protein	0.32
MM_1087	ABC transporter, ATP-binding protein	0.20
MM_1088	ABC transporter, ATP-binding protein	-0.23
MM_1096	Thermosome, gamma su	0.51
MM_1098	Conserved protein	-0.81
MM_1100	Hypothetical protein	0.11
MM_1101	Archaeosine tRNA-ribosyltransferase	0.48
MM_1104	Oxidoreductase (flavoprotein)	-0.30
MM_1105	Metallo cofactor biosynthesis protein moaA/ nifB /pqqE family	0.50
MM_1106	Putative phosphoserine phosphatase	-0.66
MM_1107	Putative phosphoserine phosphatase	-0.87
MM_1108	F420-dependent methylenetetrahydromethanopterin dehydrogenase	0.48
MM_1110	Conserved protein	0.26
MM_1111	Thymidylate kinase	0.11
MM_1114	Conserved protein	0.35
MM_1115	Conserved protein	0.22
MM_1116	Hypothetical protein	-0.05
MM_1119	Conserved protein	0.20
MM_1120	Conserved protein	-0.15
MM_1121	Glycosyl transferase	-0.63
MM_1122	Oxidoreductase (hypothetical)	0.17
MM_1123	Hypothetical protein	-0.01
MM_1124	O-antigen translocase	0.04
MM_1125	Conserved protein	-0.98
MM_1127	Glycosyl transferase	1.31
MM_1129	Conserved protein	-0.16
MM_1130	Hypothetical protein	0.17
MM_1132	UDP-glucose 6-dehydrogenase	-0.25
MM_1133	UTP--glucose-1-phosphate uridylyltransferase	0.30
MM_1134	UDP-glucose 4-epimerase	-0.26
MM_1135	Transporter	-0.33
MM_1137	Glycosyl transferase	-0.51
MM_1138	Glycosyl transferase	-0.57
MM_1139	Dolichyl-phosphate mannose synthase related protein	-0.06
MM_1140	Dolichyl-phosphate mannose synthase related protein	-0.09
MM_1141	Glycosyl transferase	-0.09
MM_1143	Glycosyl transferase	0.00
MM_1145	Conserved protein	0.13

MM_1147	Dolichol-phosphate mannosyltransferase	0.17
MM_1148	Conserved protein	-0.08
MM_1149	Conserved protein	-0.69
MM_1151	Acetyltransferase	-0.44
MM_1152	Aspartate aminotransferase	-1.29
MM_1153	Myo-inositol 2-dehydrogenase	-1.14
MM_1154	NDP-N-acetyl-D-galactosaminuronic acid dehydrogenase	-1.48
MM_1155	Conserved protein	-0.37
MM_1156	O-antigen translocase	-1.06
MM_1157	Conserved protein	0.28
MM_1158	Conserved protein	0.11
MM_1159	Hypothetical protein	0.53
MM_1161	Conserved protein	-0.29
MM_1165	Putative glycosyl hydrolase	-0.17
MM_1180	Oxidoreductase (hypothetical)	-0.47
MM_1185	Conserved protein	-0.12
MM_1198	Transposase	-0.21
MM_1201	Dihydrodipicolinate synthase	-0.15
MM_1202	Dihydrodipicolinate reductase	-0.36
MM_1204	Conserved protein	-0.72
MM_1206	Conserved protein	-0.87
MM_1213	Aspartate carbamoyltransferase, catalytic su	0.60
MM_1214	Conserved protein	0.30
MM_1215	Hexulose-6-phosphate synthase	-0.60
MM_1216	Conserved protein	1.14
MM_1218	GMP synthase [glutamine-hydrolyzing]	-0.06
MM_1219	Fe-S oxidoreductase	-0.62
MM_1220	Acetylglutamate kinase	-0.25
MM_1221	Chromosomal protein MC1	0.50
MM_1222	Conserved protein	-0.18
MM_1227	Glutamyl-tRNA (Gln) amidotransferase, su A	-0.38
MM_1228	Glutamyl-tRNA (Gln) amidotransferase, su B	-0.45
MM_1229	Nitrilase	0.43
MM_1236	Protease HTPX	-0.24
MM_1238	3-Phosphoshikimate 1-carboxyvinyltransferase	-0.13
MM_1239	Methyltransferase	0.09
MM_1240	Methyl-coenzyme M reductase, alpha su	-0.45
MM_1241	Methyl-coenzyme M reductase, gamma su	-0.34
MM_1242	Methyl-coenzyme M reductase, operon protein C	-0.33
MM_1243	Methyl-coenzyme M reductase, operon protein D	-0.35
MM_1244	Methyl-coenzyme M reductase, beta su	-0.05
MM_1245	Conserved protein	0.59

MM_1247	Conserved protein	-0.18
MM_1249	Ribulose bisphosphate carboxylase large chain	0.08
MM_1251	Cation transporter	0.03
MM_1252	Conserved protein	0.29
MM_1253	Cation transporter	0.34
MM_1254	Putative heat shock protein	1.16
MM_1255	Putative heat shock protein	1.41
MM_1261	Conserved protein	0.11
MM_1262	Xanthine-guanine phosphoribosyltransferase	-0.08
MM_1267	Signal recognition particle, su FFH/SRP54	-0.11
MM_1270	GMP synthase [glutamine-hydrolyzing]	-0.26
MM_1271	2-Dehydro-3-desoxyphosphoheptanote aldolase	-0.83
MM_1272	3-Dehydroquinate synthase	-0.95
MM_1273	3-Dehydroquinate dehydratase	-0.78
MM_1274	Shikimate 5-dehydrogenase	-0.43
MM_1275	Prephenate dehydrogenase	-0.87
MM_1276	Hypothetical protein	-0.06
MM_1277	Methyltransferase	0.52
MM_1278	Triosephosphate isomerase	-0.14
MM_1279	Orotidine 5'-phosphate decarboxylase family protein	-0.49
MM_1280	Endonuclease III	0.13
MM_1282	Conserved protein	0.38
MM_1283	Bacterioferritin	0.56
MM_1284	2-Isopropylmalate synthase	0.76
MM_1285	Sec-independent transport protein TatD	-0.36
MM_1292	Archaeosine tRNA-ribosyltransferase	-0.19
MM_1293	Ribosomal protein S18 alanine acetyltransferase	1.62
MM_1294	Conserved protein	1.78
MM_1295	DNA primase	-0.57
MM_1296	NADH oxidase	-0.60
MM_1297	RNA 3'-terminal phosphate cyclase	-0.58
MM_1298	Putative tRNA 2'phosphotransferase	0.14
MM_1299	Replication factor-A protein	-0.14
MM_1300	Transposase	0.52
MM_1301	Conserved protein	-0.16
MM_1302	Conserved protein	-0.31
MM_1303	Conserved protein	1.24
MM_1304	Conserved protein	1.73
MM_1305	Conserved protein	1.57
MM_1306	HTH DNA-binding protein	-0.72
MM_1312	Conserved protein	0.04
MM_1316	Sodium/proline symporter	0.22

MM_1321	Formylmethanofuran--tetrahydromethanopterin formyltransferase	0.15
MM_1322	Chemotaxis protein CheD	-0.55
MM_1323	Chemotaxis protein CheC	-0.25
MM_1324	Chemotaxis protein methyltransferase CheR	-0.22
MM_1325	Chemotaxis protein CheA	0.04
MM_1326	Chemotaxis receptor methylesterase, CheB	0.36
MM_1327	Chemotaxis protein CheY	0.04
MM_1329	Methyl-accepting chemotaxis protein	-0.21
MM_1334	Zinc ABC transporter, ATP-binding protein	0.06
MM_1335	Zinc ABC transporter, permease protein	0.14
MM_1336	Hypothetical protein	-1.06
MM_1337	Hypothetical protein	-0.31
MM_1339	Pyruvate:ferredoxin oxidoreductase, beta su	1.22
MM_1340	Pyruvate:ferredoxin oxidoreductase, alpha su	1.11
MM_1342	Pyruvate:ferredoxin oxidoreductase, gamma su	0.45
MM_1346	Fe-S oxidoreductase	0.32
MM_1348	Arginyl-tRNA synthetase	-1.05
MM_1349	Conserved protein	0.76
MM_1353	Conserved protein	0.27
MM_1355	Conserved protein	-0.68
MM_1357	Tram domain protein	-2.00
MM_1358	Putative methyltransferase	-1.60
MM_1359	Hypothetical protein	-0.48
MM_1362	ABC transporter, periplasmic binding protein	-2.28
MM_1364	S-layer protein	0.10
MM_1365	Thiamin-monophosphate kinase	-0.31
MM_1367	Anaerobic ribonucleoside-triphosphate reductase	0.34
MM_1369	DNA repair protein	-0.13
MM_1370	Myo-inositol-1-phosphate synthase	-0.10
MM_1371	Archaeal protein Translation Initiation Factor 2B su 1 (aIF-2B1)	-0.74
MM_1372	Hypothetical protein	0.47
MM_1375	Polyphosphate kinase	0.71
MM_1376	Exopolyphosphatase	0.17
MM_1378	Origin recognition complex su	0.75
MM_1379	Thermosome, alpha su	0.24
MM_1383	Phenylalanyl-tRNA synthetase, alpha chain	-0.19
MM_1385	Conserved protein	0.12
MM_1386	Hypothetical protein	-0.50
MM_1391	Survival protein	0.60
MM_1393	Molybdenum cofactor biosynthesis protein A	-0.65
MM_1394	Cobyrinic acid a,c-diamide synthase	-0.04

MM_1396	Conserved protein	0.18
MM_1397	DNA polymerase sliding clamp	-0.10
MM_1399	MutT-like protein	-0.29
MM_1401	Hypothetical protein	0.13
MM_1402	Phosphatidylserine decarboxylase	-0.07
MM_1403	CDP-diacylglycerol--serine O-phosphatidyltransferase	-0.18
MM_1404	Conserved protein	-0.37
MM_1405	Histidinol-phosphate aminotransferase	-0.65
MM_1407	Conserved protein	-0.36
MM_1409	Hypothetical protein	0.55
MM_1412	Hypothetical protein	0.34
MM_1415	Conserved protein	-0.18
MM_1416	Phosphoribosylformylglycinamidine cyclo-ligase	-0.33
MM_1418	3-Phosphonopyruvate decarboxylase	-0.35
MM_1419	Small heat shock protein	-0.21
MM_1420	Hypothetical protein	-0.14
MM_1421	Conserved protein	-0.02
MM_1422	Hypothetical protein	0.01
MM_1423	dCMP deaminase	-0.26
MM_1424	Protein translocase, su SecF	-0.03
MM_1425	Protein translocase, su SecD	0.19
MM_1430	Transposase	-0.09
MM_1436	Monomethylamine:corrinoid methyltransferase MtmB1	-2.24
MM_1438	Monomethylamine corrinoid protein MtmC1	-1.32
MM_1441	Inorganic pyrophosphatase	-1.58
MM_1443	PylC (Pyrrolysine synthesis)	-1.16
MM_1445	Lysly-tRNA synthetase (PylS)	0.13
MM_1448	Hypothetical protein	0.09
MM_1449	Conserved protein	0.21
MM_1454	Universal stress protein	0.03
MM_1456	Universal stress protein	0.20
MM_1457	Conserved protein	-0.16
MM_1458	Conserved protein	0.09
MM_1460	Conserved protein	-1.72
MM_1464	Hypothetical protein	0.27
MM_1471	Tryptophanyl-tRNA synthetase	-0.70
MM_1474	Metallo cofactor biosynthesis protein moaA/ nifB /pqqE family	0.12
MM_1475	Probable translation initiation factor 2 beta su	-0.35
MM_1476	LSU ribosomal protein L10E	-0.03
MM_1486	Alanyl-tRNA synthetase	-0.17
MM_1493	Conserved protein	0.11
MM_1498	Hypothetical protein	-0.26

MM_1499	Methionine aminopeptidase	-0.36
MM_1500	Xaa-Pro aminopeptidase	0.07
MM_1502	S-adenosylmethionine synthetase	0.01
MM_1504	Phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	-0.49
MM_1505	Imidazoleglycerol-phosphate dehydratase	-0.64
MM_1506	FdhD protein	0.39
MM_1514	Hypothetical protein	0.99
MM_1517	Cysteine desulfurase	-0.29
MM_1519	Amino-acid acetyltransferase	-0.17
MM_1521	Phosphoglucomutase/phosphomannomutase	-0.13
MM_1522	Putative FeS oxidoreductase	0.01
MM_1523	Zinc metalloprotease	0.09
MM_1524	Conserved protein	0.72
MM_1526	4-Hydroxybenzoate decarboxylase	-0.02
MM_1527	Citrate (si) synthase	-0.21
MM_1528	Aconitate hydratase	-0.40
MM_1538	Replication factor c su	0.32
MM_1539	Hypothetical protein	0.83
MM_1549	Sodium/proline symporter	-0.59
MM_1551	Hypothetical protein	0.57
MM_1552	Hypothetical protein	0.26
MM_1553	TRANSPORTER, RND superfamily	0.19
MM_1554	Transcriptional regulator	-0.12
MM_1555	Putative metal dependent hydrolase	-0.30
MM_1557	Signal recognition particle SEC65 su	-0.17
MM_1558	Hypothetical protein	0.02
MM_1559	Hypothetical protein	0.35
MM_1564	Molybdenum formylmethanofuran dehydrogenase, su	-0.90
MM_1567	Molybdenum formylmethanofuran dehydrogenase, su	-1.43
MM_1568	Molybdenum formylmethanofuran dehydrogenase, su	-1.44
MM_1569	Molybdenum formylmethanofuran dehydrogenase, su	-0.91
MM_1570	Conserved protein	0.14
MM_1571	Conserved protein	0.30
MM_1574	Conserved protein	-0.79
MM_1581	Putative NAD(P)H oxidoreductase	-0.43
MM_1585	Iron-sulfur flavoprotein	1.18
MM_1588	Surface layer protein (putative)	-0.10
MM_1589	Surface layer protein (putative)	-0.01
MM_1591	Conserved protein	-0.72
MM_1593	Probable RNA processing protein	0.33
MM_1595	Conserved protein	-0.91
MM_1598	Conserved protein	-0.25

MM_1603	Conserved protein	-0.03
MM_1604	Tungsten formylmethanofuran dehydrogenase su E	0.07
MM_1606	Archaeal protein Translation Initiation Factor 2B su 2aIF-2B2	0.28
MM_1607	Acetyltransferase	-0.05
MM_1608	ABC transporter, ATP-binding protein	0.27
MM_1611	Uridylate kinase	-0.62
MM_1615	Oxidoreductase, aldo/keto family	0.21
MM_1618	Aspartate-semialdehyde dehydrogenase	-0.38
MM_1619	Ferredoxin	0.30
MM_1621	Cobalamin biosynthesis protein CobW	-0.38
MM_1625	Conserved protein	-0.17
MM_1626	Conserved protein	-0.26
MM_1627	Fructose-bisphosphate aldolase	0.02
MM_1628	Deoxycytidine triphosphate deaminase	0.07
MM_1629	Conserved protein	0.32
MM_1632	Iron-sulfur flavoprotein	0.61
MM_1634	Transcriptional regulator	0.22
MM_1647	Methanol:corrinoid methyltransferase MtaB1	0.27
MM_1649	Conserved protein	-0.50
MM_1650	Conserved protein	-0.17
MM_1658	methyl-accepting chemotaxis protein	0.15
MM_1661	Type I restriction-modification system restriction su	0.26
MM_1663	Conserved protein	0.12
MM_1671	Hypothetical sensory transduction histidine kinase	0.77
MM_1675	Conserved protein	-0.42
MM_1676	Conserved protein	-0.57
MM_1681	CbiD protein	-0.41
MM_1682	DNA mismatch repair protein	-0.24
MM_1687	Dimethylamine corrinoid protein MtbC1	0.55
MM_1689	Trimethylamine:corrinoid methyltransferase MttB1	0.29
MM_1693	Dimethylamine:corrinoid methyltransferase MtbB1	-2.84
MM_1694	Dimethylamine:corrinoid methyltransferase MtbB1	-2.47
MM_1695	Conserved protein	0.41
MM_1696	Lysyl-tRNA synthetase, class I	0.16
MM_1697	LysM	0.65
MM_1702	Conserved protein	0.90
MM_1703	Conserved protein	-0.21
MM_1704	Histidine biosynthesis protein	0.13
MM_1705	Conserved protein	0.45
MM_1706	Protein-L-isoaspartate O-methyltransferase	-0.29
MM_1707	Conserved protein	0.03
MM_1712	Chorismate synthase	0.16

MM_1713	Conserved protein	-0.08
MM_1716	Transcriptional regulator, ArsR family	0.84
MM_1717	Conserved protein	-0.25
MM_1722	Hypothetical protein	-0.56
MM_1726	Heme biosynthesis protein	-0.18
MM_1728	Heme biosynthesis protein	-0.11
MM_1732	Transcriptional regulator, ArsR family	0.70
MM_1733	Metalloproteinase	-0.23
MM_1734	Conserved protein	-1.05
MM_1735	Transposase (N-terminal domain)	0.37
MM_1737	Heme biosynthesis protein	-0.02
MM_1738	Heme biosynthesis protein	-0.31
MM_1739	Heme biosynthesis protein	-0.31
MM_1741	Glutamyl-tRNA reductase	-0.30
MM_1742	Delta-aminolevulinic acid dehydratase	-0.30
MM_1743	Glutamate-1-semialdehyde 2,1-aminomutase	0.04
MM_1746	Dihydroorotate dehydrogenase electron transfer su	0.03
MM_1747	2-Hydroxy-2,4-diene-1,7-dioate isomerase	-0.11
MM_1748	Conserved protein	-0.28
MM_1749	Glutamyl-tRNA synthetase	-0.55
MM_1752	Conserved protein	-0.06
MM_1753	D-3-phosphoglycerate dehydrogenase	-0.89
MM_1755	LSU ribosomal protein L18E	0.07
MM_1756	LSU ribosomal protein L13P	-0.56
MM_1757	SSU ribosomal protein S9P	-0.45
MM_1761	Conserved protein	1.04
MM_1762	Mevalonate kinase	0.74
MM_1763	Archaeal kinase	0.79
MM_1764	Isopentenyl-diphosphate delta-isomerase	-0.13
MM_1766	Zn-dependent hydrolase	-0.16
MM_1767	Geranyltranstransferase	-0.03
MM_1768	Hypothetical protein	-0.36
MM_1770	Pyruvate, phosphate dikinase	-0.98
MM_1772	Transcription initiation factor IIB	-0.17
MM_1773	Conserved protein	-0.02
MM_1776	Thiol-specific antioxidant protein	-0.20
MM_1780	Hypothetical protein	0.26
MM_1782	Sodium/glutamate symport carrier protein	0.47
MM_1784	Hypothetical protein	0.42
MM_1787	Transposase	-0.49
MM_1788	HTH DNA-binding protein	0.12
MM_1790	Conserved protein	1.41

MM_1791	Conserved protein	0.86
MM_1795	Iron-sulfur cluster-binding protein	0.43
MM_1797	10 kDa chaperonin	0.45
MM_1801	Conserved protein	0.07
MM_1802	Aspartate aminotransferase	-0.03
MM_1804	Rubrerythrin	0.58
MM_1805	Conserved protein	0.12
MM_1807	Protein translation initiation factor 2 su alpha IF-2a	-0.28
MM_1808	SSU ribosomal protein S27E	-0.01
MM_1809	LSU ribosomal protein L44E	-0.77
MM_1810	Conserved protein	0.38
MM_1811	DNA primase small su	0.57
MM_1813	Conserved protein	-0.32
MM_1816	Conserved protein	0.44
MM_1817	Conserved protein	1.23
MM_1818	Conserved protein	0.12
MM_1821	Replication factor c su	0.34
MM_1824	Ferredoxin	0.14
MM_1825	DNA binding protein	0.33
MM_1827	Pyruvate carboxylase (biotin-containing) su B	-0.18
MM_1829	Biotin-[acetyl-CoA-carboxylase] synthetase/biotin operon repressor	0.19
MM_1830	Conserved protein	0.57
MM_1832	Transposase	0.38
MM_1833	Phosphoserine phosphatase	-0.21
MM_1835	Conserved protein	-0.10
MM_1836	Cell division control protein (MCM family)	-0.19
MM_1837	RNA methylase	0.02
MM_1839	Conserved protein	0.38
MM_1840	Conserved protein	-0.07
MM_1843	Heterodisulfide reductase, su HdrE	0.00
MM_1844	Heterodisulfide reductase, su HdrD	0.09
MM_1845	Conserved protein	0.56
MM_1846	Conserved protein	0.34
MM_1847	Hypothetical protein	-0.02
MM_1850	RNase P	1.00
MM_1853	Conserved protein	-0.49
MM_1856	Dipeptide/ oligopeptide ABC transporter, ATP-binding protein	0.17
MM_1857	Dipeptide/ oligopeptide ABC transporter, permease	0.85
MM_1858	Dipeptide/ oligopeptide ABC transporter, permease	0.88
MM_1862	Transporter	-0.12
MM_1870	Conserved protein	-0.34

MM_1871	Vanillate decarboxylase protein	0.54
MM_1872	Conserved protein	-0.16
MM_1873	Phosphohydrolase	0.62
MM_1875	Conserved protein	0.77
MM_1876	Purine phosphoribosyltransferase	-0.10
MM_1878	Methyltransferase	-0.48
MM_1879	putative RNA-binding protein	-0.57
MM_1881	Conserved protein	0.38
MM_1883	Conserved protein	0.90
MM_1885	Diaminopimelate decarboxylase	-0.71
MM_1888	Transposase	0.34
MM_1896	Phenylacetic acid degradation protein	0.02
MM_1897	Hypothetical protein	-0.90
MM_1909	Glutathione-regulated potassium-efflux system protein	-0.03
MM_1911	Cysteinyl-tRNA synthetase	0.46
MM_1913	ATP-dependent protease La	-0.07
MM_1914	Hypothetical protein	-0.12
MM_1916	Lysyl-tRNA synthetase, class II	-0.91
MM_1918	Hypothetical protein	0.06
MM_1920	Nucleotidyltransferase	0.24
MM_1922	Hypothetical protein	0.41
MM_1925	hypothetical protein	0.01
MM_1929	Hypothetical protein	-2.41
MM_1936	Conserved protein	0.08
MM_1939	Glutamine-binding protein	-0.21
MM_1944	Transporter	-0.65
MM_1949	Methyltransferase	-0.36
MM_1957	Hypothetical transcriptional regulatory protein	-0.24
MM_1958	Hit-like protein, involved in cell-cycle regulation	0.07
MM_1960	Conserved protein	-0.02
MM_1961	Hypothetical protein	0.80
MM_1962	Conserved protein	0.14
MM_1963	Tyrosyl-tRNA synthetase	0.01
MM_1964	hypothetical protein	-0.17
MM_1966	Malate dehydrogenase	-1.06
MM_1967	Hypothetical protein	-0.17
MM_1968	Cupin-type phosphoglucose isomerases	-0.21
MM_1969	Hypothetical protein	0.57
MM_1970	Single-stranded-DNA-specific exonuclease RecJ	0.52
MM_1971	Conserved protein	0.62
MM_1974	Histone acetyltransferase (ELP3 faminly)	-0.37
MM_1975	Conserved protein	-0.10

MM_1976	Conserved protein	0.23
MM_1977	Conserved protein	-0.30
MM_1980	Tungsten formylmethanofuran dehydrogenase su B	-0.04
MM_1981	Tungsten formylmethanofuran dehydrogenase su D	0.15
MM_1982	Alkyl sulfatase	0.33
MM_1984	Hypothetical protein	0.05
MM_1985	Hypothetical protein	0.21
MM_1986	Regulatory protein (putative)	0.49
MM_1995	Cobalt ABC transporter, permease protein CbiQ	-0.42
MM_1998	Cobalamin biosynthesis protein CobN	-0.82
MM_1999	Hypothetical protein	-0.63
MM_2002	Conserved protein	-0.11
MM_2003	Magnesium-chelatase su	-0.37
MM_2005	Phosphate-binding protein	-0.84
MM_2010	Phosphate transport system protein	-0.15
MM_2012	Putative RNA-binding protein	-0.13
MM_2014	Translation initiation factor 1A (EIF-1A)	0.11
MM_2017	Conserved protein	-0.72
MM_2018	Conserved protein	0.20
MM_2020	TWITCHING MOBILITY (PilT) related protein	0.06
MM_2022	TRANSPORTER, LysE family	-0.32
MM_2025	Hypothetical protein	0.55
MM_2026	Hypothetical protein	-0.02
MM_2028	Hypothetical protein	0.11
MM_2029	Conserved protein	1.21
MM_2030	Amidotransferase hisH	-0.21
MM_2032	Nodulation protein	-0.23
MM_2033	Stomatin-like protein	0.05
MM_2034	Conserved protein	-0.37
MM_2035	Orotate phosphoribosyltransferase	0.01
MM_2036	Conserved protein	-0.14
MM_2039	SSU ribosomal protein S8E	-0.60
MM_2041	Aspartate aminotransferase	-0.57
MM_2042	Archaeal transcriptional regulator	-0.09
MM_2043	Hypothetical protein	-0.45
MM_2044	Conserved protein	-0.61
MM_2052	Dimethylamine corrinoid protein MtbC2	-1.55
MM_2053	Conserved protein	-0.28
MM_2054	ABC transporter, ATP-binding protein	-0.69
MM_2055	ABC transporter, ATP-binding protein	-0.70
MM_2056	AdoCbi-P nucleotidyltransferase CobY	-0.83
MM_2057	Cobalamin [5'-phosphate] synthase CobS	-0.43

MM_2063	Conserved protein	0.45
MM_2066	SSU ribosomal protein S15P	-0.75
MM_2067	Ferric enterobactin ABC transporter, ATP-binding protein (FepC)	0.44
MM_2069	Ferric enterobactin ABC transporter, solute binding protein (FepB)	0.49
MM_2070	Nicotinate-nucleotide pyrophosphorylase	0.20
MM_2073	Quinolinate synthetase A	-0.41
MM_2076	S-adenosylmethionine synthetase	-1.98
MM_2077	Conserved protein	0.67
MM_2078	Conserved protein	0.46
MM_2083	Orotidine 5'-phosphate decarboxylase	-0.59
MM_2092	CODH nickel-insertion accessory protein (iron-sulfur protein)	-0.12
MM_2093	Indolepyruvate oxidoreductase, su	0.34
MM_2095	Hypothetical protein	0.47
MM_2099	Hypothetical protein	0.31
MM_2101	Heteropolysaccharide repeat unit export protein	-0.36
MM_2106	Putative glycosyltransferase	-0.17
MM_2108	Conserved protein	0.34
MM_2109	Glycosyl transferase	0.26
MM_2115	Protease HTPX homolog	0.25
MM_2117	Hypothetical protein	0.69
MM_2118	Hypothetical protein	-0.29
MM_2119	Hypothetical protein	-1.25
MM_2124	LSU ribosomal protein L3P	-0.95
MM_2125	LSU ribosomal protein L4	-1.10
MM_2126	LSU ribosomal protein L23P	-1.32
MM_2127	LSU ribosomal protein L2P	-1.46
MM_2128	SSU ribosomal protein S19P	-1.55
MM_2130	SSU ribosomal protein S3P	-1.60
MM_2132	RNaseP suP29	-1.45
MM_2133	SSU ribosomal protein S17P	-1.89
MM_2134	LSU ribosomal protein L14P	-2.02
MM_2135	LSU ribosomal protein L24P	-2.06
MM_2136	SSU ribosomal protein S4E	-1.74
MM_2137	LSU ribosomal protein L5P	-1.90
MM_2138	SSU ribosomal protein S14P	-1.65
MM_2139	SSU ribosomal protein S8P	-1.51
MM_2140	LSU ribosomal protein L6P	-1.95
MM_2141	LSU ribosomal protein L32E	-1.96
MM_2142	LSU ribosomal protein L19E	-2.22
MM_2143	LSU ribosomal protein L18P	-1.82
MM_2144	SSU ribosomal protein S5P	-1.85

MM_2145	LSU ribosomal protein L30P	-1.37
MM_2146	LSU ribosomal protein L15P	-1.57
MM_2148	Adenylate kinase	-0.92
MM_2149	Phosphoribosylaminoimidazole carboxylase	-1.47
MM_2152	Hypothetical protein	-1.77
MM_2155	SSU ribosomal protein S13P	-0.99
MM_2156	SSU ribosomal protein S4P	-1.22
MM_2163	Hydrogenase expression/formation protein	-0.49
MM_2171	F420-nonreducing hydrogenase II, cytochrome b su (VhtC)	-0.38
MM_2181	Fructose-1,6-bisphosphatase	-0.49
MM_2182	Hypothetical protein	-1.14
MM_2183	Hypothetical protein	-0.82
MM_2192	Transposase	0.25
MM_2196	Conserved protein	1.06
MM_2203	Hypothetical protein	-0.40
MM_2205	Ribose-phosphate pyrophosphokinase	-0.03
MM_2209	Conserved protein	0.04
MM_2210	Oligosaccharyl transferase	-0.40
MM_2211	Hypothetical protein	0.08
MM_2213	Putative glycosyltransferase	-0.52
MM_2217	Conserved protein	0.69
MM_2224	Trp repressor binding protein	-0.79
MM_2225	Glucoamylase	0.21
MM_2230	Transposase	0.01
MM_2232	Transposase	0.02
MM_2235	Conserved protein	-0.92
MM_2237	Transcriptional regulator, ArsR family	0.75
MM_2243	Putative methyltransferase	0.97
MM_2263	SSU ribosomal protein S10P	-0.59
MM_2264	Protein translation elongation factor 1A (EF-1A)	-0.84
MM_2265	Protein translation elongation factor 2	-1.12
MM_2266	SSU ribosomal protein S7P	-1.52
MM_2267	SSU ribosomal protein S12P	-1.45
MM_2268	NusA protein homolog	-0.50
MM_2269	LSU ribosomal protein L30E	-1.22
MM_2270	DNA-directed RNA polymerase, su A''	-0.80
MM_2271	DNA-directed RNA polymerase, su A'	-1.05
MM_2272	DNA-directed RNA polymerase, su B''	-1.43
MM_2273	DNA-directed RNA polymerase, su B'	-0.81
MM_2274	DNA-directed RNA polymerase, su H	-1.06
MM_2275	Hypothetical sensory transduction histidine kinase	-0.54
MM_2276	Hypothetical sensory transduction histidine kinase	-0.02

MM_2278	Adenosylhomocysteinase	-0.58
MM_2279	Chlorohydrolase family protein	-0.19
MM_2280	Conserved protein	-0.14
MM_2281	Hypothetical protein	-0.15
MM_2283	DNA/pantothenate metabolism flavoprotein	0.46
MM_2294	Type I restriction-modification system methylation su	-0.03
MM_2299	Hypothetical protein	0.64
MM_2303	Transcriptional regulator	-0.10
MM_2306	L-asparaginase	-0.17
MM_2309	Hypothetical protein	0.25
MM_2312	F420-nonreducing hydrogenase I, cytochrome b su (VhoC)	0.18
MM_2313	F420-nonreducing hydrogenase I, large su (VhoA)	-0.02
MM_2314	F420-nonreducing hydrogenase I, small su (VhoG)	-0.40
MM_2316	Hydrogenase expression/formation protein	0.18
MM_2317	Hydrogenase expression/formation protein	-0.17
MM_2320	Ech Hydrogenase, su A	-0.09
MM_2321	Ech Hydrogenase, su B	-0.19
MM_2322	Ech Hydrogenase, su C	-0.39
MM_2323	Ech Hydrogenase, su D	-0.23
MM_2324	Ech Hydrogenase, su E	-0.61
MM_2325	Ech Hydrogenase, su F	-0.30
MM_2326	COP associated protein	0.54
MM_2328	Copper-exporting ATPase	-0.39
MM_2331	Uncharacterized permease	-0.27
MM_2333	Multidrug efflux pump	0.76
MM_2335	Conserved protein	-0.55
MM_2337	Conserved protein	-0.91
MM_2338	Conserved protein in Methanosarcina spec.	-0.15
MM_2341	Succinate-semialdehyde dehydrogenase [NADP+]	0.23
MM_2342	Hypothetical protein	0.28
MM_2346	Methyltransferase	0.11
MM_2348	O-linked N-acetyglucosamine transferase	-0.31
MM_2350	O-linked N-acetyglucosamine transferase	0.67
MM_2352	Conserved protein	0.92
MM_2354	Thioredoxin	0.15
MM_2357	Hypothetical protein	-0.53
MM_2358	6-Phosphofructokinase (ADP)	-0.57
MM_2359	Conserved protein	-0.61
MM_2360	Phosphoribosylaminoimidazole carboxylase, catalytic su	-0.13
MM_2362	Shikimate kinase	0.43
MM_2364	GTP-binding protein	-0.25
MM_2365	Conserved protein	-0.31

MM_2366	Conserved protein	-0.22
MM_2367	Hexulose-6-phosphate isomerase	-0.04
MM_2368	Aspartate aminotransferase	0.21
MM_2369	Cytochrome c-type biogenesis protein	1.62
MM_2370	GTP-binding protein	-0.81
MM_2374	Conserved protein	0.18
MM_2376	Conserved protein	0.36
MM_2378	Conserved transmembrane protein	0.28
MM_2379	5'-Methylthioadenosine phosphorylase	0.05
MM_2382	Hypothetical protein	0.12
MM_2383	Small nuclear riboprotein (snRNA) homolog	-0.19
MM_2384	Conserved protein	-0.29
MM_2387	Cobalt transport ATP-binding protein CbiO	-0.08
MM_2388	Cobalt ABC transporter, permease protein CbiQ	0.45
MM_2393	Conserved protein	-0.02
MM_2394	Transcriptional regulator, MerR family	0.00
MM_2396	Heme exporter, protein C	0.51
MM_2397	Heme exporter, protein B	0.06
MM_2398	ABC transporter, ATP-binding protein	0.47
MM_2399	Peptide methionine sulfoxide reductase	-0.39
MM_2400	Hypothetical protein	0.27
MM_2401	Conserved protein	0.68
MM_2403	Potassium channel protein	0.56
MM_2405	Cation transporter	0.27
MM_2406	Conserved protein	0.31
MM_2409	Conserved protein	1.07
MM_2415	Hypothetical protein	0.01
MM_2419	DNA gyrase, su B	-0.52
MM_2420	DNA gyrase, su A	-0.54
MM_2421	Chaperone protein	-0.23
MM_2428	Methylthiol:coenzyme M methyltransferase MtsB	0.57
MM_2431	Conserved protein	0.38
MM_2432	Putative pyridoxine biosynthesis protein	-0.90
MM_2435	Hypothetical sensory transduction histidine kinase	-0.42
MM_2438	Hypothetical protein	0.36
MM_2442	Conserved protein	-0.03
MM_2445	Conserved protein	-0.28
MM_2446	Transcriptional regulator, ArsR family	0.18
MM_2448	Transposase	0.62
MM_2449	Conserved protein	-0.02
MM_2452	Conserved protein	-0.71
MM_2454	ABC transporter, permease protein	-0.58

MM_2457	Dipeptide/oligopeptide transporter, permease protein	0.23
MM_2458	Dipeptide/oligopeptide transporter, permease protein	0.11
MM_2459	Methyltransferase	0.50
MM_2460	Dipeptide/oligopeptide-binding protein	0.12
MM_2464	Nucleoside diphosphate kinase	-0.76
MM_2466	SSU ribosomal protein S28E	-0.66
MM_2470	Surface layer protein (putative)	-0.36
MM_2471	Hypothetical protein	-0.35
MM_2478	Chloride channel (putative)	-0.73
MM_2479	F420H2 dehydrogenase, suO	0.87
MM_2480	F420H2 dehydrogenase, suN	0.76
MM_2481	F420H2 dehydrogenase, suM	0.83
MM_2482	F420H2 dehydrogenase, suL	0.43
MM_2483	F420H2 dehydrogenase, suK	0.80
MM_2486	F420H2 dehydrogenase, suI	0.74
MM_2487	F420H2 dehydrogenase, suH	0.91
MM_2488	F420H2 dehydrogenase, suD	0.96
MM_2489	F420H2 dehydrogenase, suC	0.69
MM_2490	F420H2 dehydrogenase, suB	0.54
MM_2491	F420H2 dehydrogenase, suA	1.05
MM_2493	Oxidoreductase (hypothetical)	0.21
MM_2494	FO synthase, su CofG	0.81
MM_2495	F420 biosynthesis protein FbiC	0.88
MM_2496	FO synthase, su CofH	0.17
MM_2497	4-Methyl-5(B-hydroxyethyl)-thiazole monophosphate synthase	0.73
MM_2499	Geranylgeranyl reductase	-0.48
MM_2502	Trk system potassium uptake protein	-0.07
MM_2503	Trk system potassium uptake protein TrkA	-0.64
MM_2504	Chaperone protein DnaJ	0.02
MM_2505	Chaperone protein	1.39
MM_2506	GrpE protein	1.19
MM_2511	Hypothetical protein	-0.88
MM_2512	1-Pyrroline-5-carboxylate synthetase	-0.33
MM_2514	Archaeal protein Translation Elongation Factor 1, su beta	-0.66
MM_2515	Hypothetical sensory transduction histidine kinase	-0.27
MM_2516	Probable transcriptional regulator	0.01
MM_2519	GTP-binding protein homolog	0.39
MM_2522	Universal stress protein	0.03
MM_2523	Hypothetical protein	0.34
MM_2524	Phage shock protein A	0.20
MM_2525	Hypothetical protein	1.75
MM_2526	Conserved protein	0.13

MM_2527	Hypothetical protein	-0.28
MM_2528	N2,N2-dimethylguanosine tRNA methyltransferase	-0.26
MM_2529	Hypothetical protein	-0.15
MM_2530	Transposase	-0.77
MM_2531	Hypothetical protein	-0.14
MM_2532	Hypothetical protein	-0.21
MM_2535	Conserved hypothetical protein	-0.76
MM_2538	Conserved hypothetical protein	-0.06
MM_2541	Cation-transporting ATPase	-0.53
MM_2542	Phosphohydrolase (MUTT/NUDIX family protein)	0.11
MM_2552	Hypothetical protein	-0.14
MM_2554	Acetyltransferase	-0.48
MM_2556	ATP-dependent RNA helicase	-0.47
MM_2557	Catalase	-0.41
MM_2559	Hypothetical protein	0.04
MM_2563	Ferredoxin	-0.10
MM_2564	Hypothetical protein	-0.56
MM_2565	Pyruvate formate-lyase activating enzyme	0.10
MM_2566	CODH nickel-insertion accessory protein	-0.24
MM_2567	ABC transporter, periplasmic binding protein	-0.13
MM_2568	ABC transporter, permease protein	0.06
MM_2569	ABC transporter, permease protein	0.00
MM_2570	ABC transporter, ATP-binding protein	-0.09
MM_2571	ABC transporter, ATP-binding protein	-0.07
MM_2572	Methyltransferase	0.15
MM_2573	Iron dependent transcriptional repressor	0.93
MM_2581	Pyruvate formate-lyase activating enzyme related protein	0.11
MM_2582	Hypothetical protein	0.08
MM_2583	Sec-independent protein translocase, protein	0.08
MM_2586	Sec-independent protein translocase, protein	0.60
MM_2593	Hypothetical protein	-0.09
MM_2594	Hypothetical protein	0.69
MM_2595	Hypothetical protein	-0.05
MM_2596	Hypothetical protein	0.53
MM_2598	Hypothetical protein	0.49
MM_2608	Nucleotidyltransferase	0.62
MM_2613	LemA protein	0.76
MM_2616	LSU ribosomal protein L15E	-1.19
MM_2622	Hypothetical protein	-0.20
MM_2623	Ribonuclease	-0.54
MM_2624	Ribonuclease	-0.95
MM_2625	LSU ribosomal protein L37AE	-0.92

MM_2628	Conserved protein	0.84
MM_2637	Hypothetical protein	-0.16
MM_2639	Conserved protein	0.40
MM_2646	Sensory Transduction protein Kinase	0.39
MM_2648	Hypothetical protein	0.10
MM_2649	Aspartate aminotransferase	-0.64
MM_2652	Hypothetical protein	0.49
MM_2653	N5,N10-methenyltetrahydromethanopterin cyclohydrolase	-0.51
MM_2656	Peptidyl-prolyl cis-trans isomerase	-0.45
MM_2657	transcriptional regulator	-0.84
MM_2659	Acetyltransferase	0.10
MM_2660	Sodium-calcium exchanger	0.73
MM_2661	Hypothetical protein	0.11
MM_2675	Serine protease inhibitor	-0.22
MM_2676	Conserved protein	0.34
MM_2679	Conserved protein	-0.12
MM_2680	Conserved protein	0.57
MM_2692	Hypothetical protein	0.05
MM_2693	Hypothetical protein	0.21
MM_2699	Transposase	0.15
MM_2704	Type I restriction-modification system methylation su	0.24
MM_2705	Type I restriction-modification system restriction su	-0.68
MM_2706	Conserved protein	0.03
MM_2709	Conserved protein	0.10
MM_2711	Conserved protein	0.11
MM_2712	Conserved protein	-0.41
MM_2713	Homoserine dehydrogenase	-0.42
MM_2721	Conserved protein	-0.29
MM_2722	Hypothetical protein	0.47
MM_2723	Phosphoenolpyruvate synthase	-0.34
MM_2731	Multiple antibiotic resistance protein	-0.52
MM_2732	Conserved protein	0.21
MM_2733	Hypothetical protein	0.21
MM_2737	Conserved protein	-0.73
MM_2742	Hypothetical protein	-0.01
MM_2744	Conserved protein	-0.05
MM_2769	Iron-containing alcohol dehydrogenase	0.39
MM_2770	Glycogen debranching enzyme	-0.12
MM_2772	Conserved protein	-0.17
MM_2774	Conserved protein	0.42
MM_2782	Glyceraldehyde 3-phosphate dehydrogenase	-0.03
MM_2783	Suppressor protein SuhB homolog	-0.01

MM_2785	2-Isopropylmalate synthase	1.12
MM_2786	Conserved protein	-0.39
MM_2788	Indolepyruvate oxidoreductase, alpha su	-0.71
MM_2789	Hypothetical protein	-0.55
MM_2792	Molybdopterin converting factor, su 2	0.69
MM_2793	Molybdopterin-guanine dinucleotide biosynthesis protein	-0.09
MM_2794	Hypothetical protein	-0.48
MM_2795	Conserved protein	-0.07
MM_2800	Conserved protein	-0.52
MM_2802	Hypothetical protein	-0.03
MM_2803	Carbon monoxide dehydrogenase accessory protein	0.29
MM_2805	Asparagine synthetase [glutamine-hydrolyzing]	-1.03
MM_2806	Hypothetical protein	-0.68
MM_2808	Conserved protein	0.89
MM_2812	Phenylalanyl-tRNA synthetase, beta chain	-0.30
MM_2816	Conserved protein	0.97
MM_2818	Anthranilate synthase, component I	-0.10
MM_2820	Anthranilate phosphoribosyltransferase	-0.42
MM_2824	Coenzyme F420-dependent glucose-6-phosphate dehydrogenase	-0.52
MM_2829	Peptidyl-prolyl cis-trans isomerase	-0.06
MM_2833	ABC transporter, ATP-binding protein	0.12
MM_2836	Enolase	0.57
MM_2842	5-Nitroimidazole antibiotic resistance protein	-0.43
MM_2845	Hypothetical protein	-0.13
MM_2848	Conserved protein	-0.21
MM_2855	ABC transporter, permease protein	0.58
MM_2859	Hypothetical protein	-0.36
MM_2860	Conserved protein	-0.72
MM_2862	Hypothetical protein	-0.66
MM_2864	Conserved protein	0.39
MM_2869	Hypothetical protein	0.23
MM_2874	Conserved protein	-0.63
MM_2877	Hypothetical protein	0.57
MM_2878	Hypothetical protein	0.46
MM_2882	Conserved protein	-0.73
MM_2884	Conserved protein	0.03
MM_2885	Metalloendopeptidases (putative)	0.32
MM_2886	Hypothetical sensory transduction histidine kinase	0.17
MM_2888	Endonuclease III	0.06
MM_2891	ABC transporter, permease protein	-0.08
MM_2893	Hypothetical protein	-0.08
MM_2900	Conserved protein	0.37

MM_2903	Transposase	0.30
MM_2910	Adenine deaminase	-0.45
MM_2913	Conserved protein	0.60
MM_2919	Hypothetical protein	0.48
MM_2925	Hypothetical protein	0.09
MM_2930	Hypothetical protein	-0.22
MM_2937	Conserved protein	-0.05
MM_2942	Conserved protein	0.05
MM_2947	Conserved protein	-0.34
MM_2949	Hypothetical protein	0.51
MM_2950	Conserved protein	0.92
MM_2951	Hypothetical protein	-0.11
MM_2952	Conserved protein	0.30
MM_2953	Transcriptional regulator	-1.33
MM_2954	Transcriptional regulator	0.14
MM_2955	Hypothetical sensory transduction histidine kinase	0.36
MM_2955	Hypothetical sensory transduction histidine kinase	0.75
MM_2956	Conserved protein	0.61
MM_2958	Conserved protein	0.33
MM_2959	Hypothetical protein	0.21
MM_2961	Dimethylamine corrinoid protein MtbC3	-2.43
MM_2962	Dimethylamine:corrinoid methyltransferase MtbB3	-2.67
MM_2965	Hypothetical sensory transduction histidine kinase	-0.38
MM_2967	Isoleucyl-tRNA synthetase	-0.61
MM_2968	Conserved protein	-0.67
MM_2972	Hypothetical protein	0.58
MM_2973	Hypothetical protein	-0.05
MM_2977	Hypothetical protein	0.03
MM_2981	Type I restriction-modification system methylation su	-0.41
MM_2984	Putative ferredoxin	-0.01
MM_2985	Transcriptional regulator, ArsR family	-0.91
MM_2989	Pyruvate formate-lyase activating enzyme	1.16
MM_2996	Hypothetical protein	-1.09
MM_2998	Transposase	0.05
MM_3004	Conserved protein	0.43
MM_3005	Acetyltransferase	-0.46
MM_3007	Probable transcriptional regulator	0.10
MM_3011	Hypothetical protein	-0.17
MM_3012	Hypothetical protein	0.53
MM_3013	ABC transporter, permease protein	0.23
MM_3014	Conserved protein	0.48
MM_3015	Hypothetical protein	0.16

MM_3016	Cobalt transport ATP-binding protein CbiO	-0.06
MM_3018	Conserved protein	-0.33
MM_3019	Conserved protein	-0.20
MM_3020	Conserved protein	0.11
MM_3021	ABC transporter, ATP-binding protein	-0.42
MM_3022	Conserved protein	-0.18
MM_3023	Conserved protein	-0.21
MM_3024	Conserved protein	-0.50
MM_3025	Hypothetical protein	-0.55
MM_3038	Transposase	0.27
MM_3041	Hypothetical protein	-0.13
MM_3042	Coenzyme F420 hydrogenase, beta su	0.11
MM_3043	Coenzyme F420 hydrogenase, gamma su	0.32
MM_3045	Coenzyme F420 hydrogenase, alpha su	0.71
MM_3065	Hypothetical protein	-0.21
MM_3066	Fumarate hydratase, alpha su	0.31
MM_3067	Fumarate hydratase, beta su	0.11
MM_3068	Conserved protein	0.14
MM_3074	RNase P RNA component	0.25
MM_3075	Hypothetical protein	0.04
MM_3076	DNA topoisomerase I	-0.36
MM_3078	mRNA 3'-end processing factor	0.48
MM_3079	Hypothetical protein	-0.03
MM_3081	Serine/threonine protein kinases	-0.08
MM_3082	Hypothetical protein	0.13
MM_3084	Protease I	0.45
MM_3086	Nicotinate phosphoribosyltransferase	0.33
MM_3088	Carbonic anhydrase	0.55
MM_3089	Hypothetical protein	1.07
MM_3093	Hypothetical protein	-0.24
MM_3094	Hypothetical protein	1.13
MM_3101	Two component system histidine kinase	-0.14
MM_3104	Hypothetical protein	-1.02
MM_3105	Probable cytosine deaminase	-0.55
MM_3107	Hypothetical protein	0.44
MM_3108	Hypothetical protein	0.44
MM_3109	Glycogen phosphorylase	-0.15
MM_3110	Hypothetical protein	0.19
MM_3111	Hypothetical protein	0.36
MM_3113	Oligoendopeptidase F	0.12
MM_3115	Hypothetical protein	0.49
MM_3116	Hypothetical protein	0.35

MM_3123	Hypothetical protein	-0.31
MM_3124	Hypothetical protein	-0.30
MM_3128	Hypothetical protein	-0.49
MM_3130	Hypothetical protein	-0.47
MM_3134	Protease HTPX	-0.54
MM_3135	ABC transporter, ATP-binding protein	-0.44
MM_3136	ABC transporter, permease protein	0.52
MM_3139	Large-conductance mechanosensitive channel	0.22
MM_3140	Flavodoxin	-0.20
MM_3141	Type I restriction-modification system restriction su	0.88
MM_3143	Hypothetical protein	0.74
MM_3148	Exodeoxyribonuclease III	-0.46
MM_3149	Hypothetical protein	0.34
MM_3150	Methyltransferase	0.02
MM_3154	Hypothetical protein	0.43
MM_3162	Hypothetical protein	-0.45
MM_3170	Transcriptional regulator, MerR family	-0.07
MM_3179	Transcriptional regulator	-0.33
MM_3183	Ketoisovalerate oxidoreductase su	-0.93
MM_3185	Ornithine decarboxylase	-2.62
MM_3187	Flavodoxin	0.51
MM_3188	Glutamine synthetase	-2.75
MM_3189	Potassium channel protein	0.55
MM_3190	Hypothetical protein	-0.59
MM_3198	Hypothetical protein	-0.43
MM_3201	Conserved protein	0.53
MM_3202	Conserved protein	0.86
MM_3213	Prismane protein	0.78
MM_3219	Conserved protein	-0.20
MM_3220	Transporter	0.13
MM_3230	Phosphate permease	-0.87
MM_3231	Conserved protein	0.09
MM_3234	Hydroxyethylthiazole kinase	-0.34
MM_3235	Thiamin-phosphate pyrophosphorylase	-0.50
MM_3248	Hypothetical protein	0.60
MM_3255	Hypothetical protein	-0.18
MM_3258	Hypothetical protein	-0.32
MM_3264	F420 hydrogenase/dehydrogenase, beta su	-0.84
MM_3270	Ferredoxin-thioredoxin reductase, catalytic chain	-0.39
MM_3271	Glutaredoxin	0.28
MM_3275	Conserved protein	1.00
MM_3278	Hypothetical Membrane Spanning protein	-0.05

MM_3282	Probable ATP-dependent helicase	-0.24
MM_3284	Transcriptional regulator	0.14
MM_3286	Hypothetical protein	0.71
MM_3287	Hypothetical protein	0.63
MM_3288	Conserved protein	-0.59
MM_3289	Excinuclease ABC, su B	-0.45
MM_3290	Excinuclease ABC, su C	-0.60
MM_3291	Excinuclease ABC, su A	-0.68
MM_3293	Iron-sulfur cluster-binding protein	0.56
MM_3303	Hypothetical protein	-0.09
MM_3304	Threonyl-tRNA synthetase	-0.56
MM_3305	Putative DNA or RNA helicase of superfamily II	-0.33
MM_3306	Conserved protein	-0.22
MM_3307	Conserved protein	-0.15
MM_3312	5-Methylcytosine-specific restriction enzyme A	-0.17
MM_3314	N-5'-phosphoribosyl)anthranilate isomerase	0.10
MM_3325	Permease, Na+/H+-dicarboxylate symporter	0.39
MM_3331	Conserved protein	-0.01
MM_3334	Monomethylamine corrinoid protein MtmC2	-1.78
MM_3335	Monomethylamine:corrinoid methyltransferase MtmB2	-1.72
MM_3336	Monomethylamine:corrinoid methyltransferase MtmB2	-2.00
MM_3338	Hypothetical protein	-0.23
MM_3339	Hypothetical protein	-0.11
MM_3342	Conserved protein	-1.10
MM_3343	Transposase	0.34
MM_3355	Conserved protein	-0.28
MM_3361	Hypothetical protein	-0.07
MM_3370	Hypothetical protein	0.16
MM_3371	Conserved protein	-0.08

Tab. 10.5: Sequences of antisense RNA probes used for northern blot analyses.
The probes were generated by PCR from genomic *M. mazei* DNA using the primers listed in Tab. 2.7.

Sequence of the probe against the 16S rRNA of *M. mazei*:

CAGAAGUCGAACCGGAUGUAUAACGACAGCGGGGGCCACUCAACAGGCCGCA
ACUCAGGUUAAUUUGGCGUCCGAGGUGGGCAACAACACGAGGGGGCGGUUA
AGGAAAUUCAAAGUCGGAACGCCGGCAUGAAGGGUCCACCGAGCGAAGUGCC
GAAGGGACGCCGUGGUCUGUGCCAGCGCGGUACGGACUGUGGAUCGCUCGU
AGCAAAUGCCGACCCUGAUGGGCCCAUAGAUUAGGCCAAGCACGGGGGUCGA
AAGCAGGGAGUGGCAGCUUGGGCAAGACCAUUCUGCGGAAGCGGUGUCCAC
CAGGGUGUCCCUAAUGUUCUAAAGUGAGGAUGGGGACAUCAUGGAGAAUGGA
GAGGGCCAAGGUUCAGACCGUCAUAGGGGGCUUUCGGAUUAUCAACUCGAUA
GUCUAAAGGGCCUCCUGACUGGUUUGGCCGAUGCCUGGGAAAUCUGGGUUA

UUAGUGCUAGUGGUGAGCCCGGCGGCCACAAUGGCGCCGCCGACCGUGGCC
AGAACGGGCCGGGAACGAUUGCCUACAUAAAAUGUGUAGGCCUGUCGGUCGU
AUACUACGACCGUGAGCCACAGGGGAAUAGUGCCAAAGGGCGUAACAUUUCA
AAAGCGCGGACGACGCGGGGCAUCCCGGACCUAAGUACAGAGUCU

Sequence of the probe against *orf* MM_1070:
UACUGGCUAUACUCGCUUAAGUGUGAAUUUUGUUCUGAAAAUCGGCGGGACU
UUCCGCUUGGACAACUGUUUCAUGGACAAACAAGGCAUUGGGUCUGGCCUUA
ACAUCUUGAGUACCUGCAUCAGCCACGGGGGACCGGACUUCGGGUGUGUUU
GGGGCUUGAGUACCGUUUCGACCGUGAGCGGUUGGUGCUCGAGUCGCCUGA
ACUUCGACAUUCUGAAGGGAUGACGGAAUGGCAAGACCAGCUUCGUUACCCG
ACACUUUAGUUGUACCCAUGGUUCUUGUCUGUCGGAAGGCAAUGGCCAGUG
GGGAUAGGGUUCCUGGAACUUCCGCGUCGUCAGGGACGUCUGGAGGACGUC
UCCCCGUCUUAAGGUCAUCAAGAACUUCGUUAAUUCUAGUAGUCCCUUUUUC
AACCUGGACUGCACGGUUAGCAACCGCCAUACCUUCCGGGACAAUGUCAGCG
UAGGCUGGAACAUUCACAGUUUAGGAAGUACUUUAC

Sequence of the probe against *orf* MM_2242:
GGUAUAUUAGUACGAACUAUCAUUUCACUUUAAGAAAUUUCGAGAGCCUCUAC
UUUGUGCCGAAUGGUAGGAGAGAAUGGACGACCUUGUGUCGAUGACACGGA
CACUGAAAAGAAGGGAAUGUUUUUUCCUGGUCUGUUGCCAAAGUUUUGUAAA
CUUUCAGGAUCAACUUCGACCUUAAAAUUUCAUGCUUUUCGUCCCUUCUUUG
CAUUAAAUGUCGUAAUUUUUGCUUCUCUAGUCGACAAACGAUAAUAGAGAACC
UUAGGCCCUAUAUUUCGGGACAACACUAUCCGUUGUCCGAAGUCCUCAUACA
AGACAAAGUCUCCAUACAGGACACACAUUUUGGCUUAUGUCUUAGUUGUUGU
GAUAAUUACUCUCAUGACUUUUGGCCCAACU

Sequence of the probe against *orf* MM_2243:
UACCUACGCUUACUUUUUUUCCUUCAAUAGUUUUUCUUCAUAGUCCUUUAAC
GUUAAAAUCCCCCGAGGACGACAAGACCGCCUACGACGCCACUAAGAUUGAG
UUGUCUGGAAAGGUCCAGUGAACCAAUAAGGCUUAGACUGCAAGUCCGACAA
GGCCUACGCUUAUACCCAGAACCGACGCCGUUAGGAUGGCGAAAACGUCUUG
ACUUUGGGCCUCUACAGCAAGACCUAGACCCAAGACCGCCCCCAAAACUAACA
AAAGAACGACGUGUCUUUUAUCCUUCGAGUCCUUUUCAAUAGCCCCAGCUAU

ACUGAGAACUUUACCAACUUUUUCGCGUCCGAUUACGGGCUUUUAUACCGAU
GAGUUUACAUCUUAAGGCGGUACCCCUAUAACUCAGAGAAGGCCAAUUUCUA
UCAAGACACCUACAAUAAUAGUCAUUGACACAAUAAUUGGACCGAGGACUAUU
CCUUUUUCAAAAGUCUCUCCGCAAGGCACAAGA

Sequence of the probe against *orf* MM_2962:
UACCGUUGGCUUAUACGAAAUUCCUACCCUCUGCCCUUUGCAUAGAAGGAAU
GAUUCCUGUUCUAGUACCUCCUCGAACUCCGUCCUUACCGCUUACGGAGACU
GGAGCCACUUUAAGGGCUGGAUUCACCGCUACUUUAGCUGUUCGAACGUCUU
UAAAACUACUACGGGCCGUUUUGGCAGUCACAGCUCGUCCCGUACCUUCAGG
GACAGUGUGUGCUGUAUCCGUGUGAUUCCGAACUGCCACUGGUCCCGUUGU
CACCACAACCAUAUGGAAGGUCGGCAGAACAGCCCACGUACUACGUGCUUUC
CCGUAAACCGCGACUGUGUUACCUUGACCCAGUGUAGCUGAUGUCGAUGUUC
GGACAUUUCGGACAGCAUCGUUUGCUCACGGUCCGUUACCUUCAGACGGUC
GUCUUGUACUAGUAAGGCGAGAAAAUGCCACGUUACGGCUUGUACCCCGACA
UGAUGUGAGGACUGCCUGGAAAGCUUUUGGGGCCUCUGGAGUACUUCCGAA
AGUUCUAGGUCCUUCGGACCCUGAGGUACCUUGUACGUCGGCGCGUGGAAU

Sequence of the probe against *orf* MM_3188:
GGUCGUUCACUUAAGUGGUUUCUACUGUAGUAGUUUAAGCAGUUUCUAUUGC
CAUAAUUCUACGACUGGAAGGCGAUGCAACCGCCUCUAUCGUCCGAGUUUCG
GGAGUCGAAGUAGUAGUCCUUGCUCCUUGUGGAGCUGUUACACGAAAGAUG
GCCUCUUUCUCACCUGCCGAGUUCGGAUAAGUUUAUGUAUCUUCGGCUGAGU
UCGCUGGAAAUACAACAGGGAUUUAUGGCGUGACGAAAACAUUUGGGCAAAC
UCCUUUAGGGAUGGGAACUGGACGAGACGAGAAUGAAACUGUUCCUGCCUUU
GGGAGAACGUUCGAGACGACUUUUGUAGUACUUCUUUCGAGUACGACAAGAC
UUCCUUCUCUGGCCCAUGCUCAAUUUGCGUUACCGACUUGAGCUUAUGAUGU
AGUAGUCGCUGUUUCUUUACUAACUGUGGCUGAAGGGACGUCAACUGGUCUC
UCCUAUGGUGCUUCGUCCGGGGAAGUGGUUCAAGCUGGUCGAGUCCUUUCU
ACGGUACGAGCGUUAACGGCUUCGG

Lebenslauf

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Erklärung:

Hiermit erkläre ich, gem. § 6 Abs. (2) f) der Promotionsordnung der Fakultäten für Biologie, Chemie und Mathematik zur Erlangung der Dr. rer. nat., dass ich das Arbeitgebiet, dem das Thema „The capability of methanoarchaea to form methyl and hydride derivatives of metals and metalloids“ zuzuordnen ist, in Forschung und Lehre vertrete und den Antrag von Herrn Frank Thomas befürworte und die Betreuung auch im Falle eines Weggangs, wenn nicht wichtige Gründe dem entgegenstehen, weiterführen werde.

Essen, den 15.04.2011 _____

Prof. Dr. Reinhard Hensel

Erklärung:

Hiermit erkläre ich, gem. § 7 Abs. (2) c) + e) der Promotionsordnung Fakultäten für Biologie, Chemie und Mathematik zur Erlangung des Dr. rer. nat., dass ich die vorliegende Dissertation selbständig verfasst und mich keiner anderen als der angegebenen Hilfsmittel bedient habe.

Essen, den 15.04.2011 _____

Frank Thomas

Erklärung:

Hiermit erkläre ich, gem. § 7 Abs. (2) d) + f) der Promotionsordnung der Fakultäten für Biologie, Chemie und Mathematik zur Erlangung des Dr. rer. nat., dass ich keine anderen Promotionen bzw. Promotionsversuche in der Vergangenheit durchgeführt habe und dass diese Arbeit von keiner anderen Fakultät/Fachbereich abgelehnt worden ist.

Essen, den 15.04.2011 _____

Frank Thomas